

University paymasters discredited

The British government's latest policy document on higher education is a disgrace. Academics now have nothing to lose but the tenure of their jobs (itself proscribed).

THE British government's latest proposals for higher education (p.270) are most of all distinguished by their vacuity, the quality of being "content-free" in the newest civil service argot. The officials at the Department of Education and Science who have laboured for the past year and more to put flesh on the bones of Sir Keith Joseph's preconceptions about higher education and those who work therein have produced an agenda for a non-discussion, for interested parties to say where they have always stood while the cause they are supposed to share is lost.

Shabby is the best word for this literally appalling document, and calculated indifference is the only way of describing the policy it embodies. Ordinary innocents might suppose that a state paper with "... higher education into the 1990s" in its title might have something valuable to say about the function of higher education in contemporary society. Carefully, the draughtsmen of this latest prospectus for British universities and polytechnics give no hostages to fortune. They simply quote the Robbins report of 1963 and launch into a discussion of the role of higher education in "the production of qualified manpower".

That calculated indifference is the British government's policy in higher education is now made crystal clear. (The calculation is that if the government does nothing about the problems of higher education, they will solve themselves just as mail that is left unanswered is usually overtaken by events, in this connection the disappearance of a nuisance.) Thus the green paper stolidly dodges the question that higher education has been asking the British government for at least five years, that there should be a clear definition of its function. (The explanation that there can be no longer forecast of the funds that will be available to higher education than for, say, the Ministry of Defence, is an answer to a different question.) Instead, the green paper parades the need that institutions of higher education should look to other sources than the British government to keep themselves in being. Perhaps universities and polytechnics should take the hint and make a dash for freedom). That way, they might be better off.

The plain truth is that the British government has systematically misled British higher education. Although the once-and-for-all reduction of 8.5 per cent of university budgets decreed in 1980, together with the manipulation of the fees of overseas students whose variable effects were to reduce university incomes by an average of 5 per cent, was accompanied by promises that there would be "level funding" once the shock had been absorbed, the fact is that the budgets of the universities as a whole are still falling by between 1.5 per cent and 2 per cent a year in real terms. The polytechnics lie some two years behind on the path to penury, chiefly because of the delay in creating their supervisory National Advisory Body. During the same period, the government has put a ceiling on the numbers of students that universities and polytechnics separately are allowed to teach, has threatened a cumbersome procedure for denying academics the right to tenure of their jobs and has generally exhorted everyone in sight to find other sources of funds than the public purse.

Nobody should deny that the government has problems. In the prosperous early 1960s, it seemed sensible that there should be a public system of higher education to which all qualified would-be students should have access, which principle would determine the scale of the system. It also then seemed financially feasible that the central government should meet the cost to local

authorities of maintenance grants for all students winning themselves a place in higher education. Even the present government's belief that British higher education is indolently unconcerned with Britain's chronic economic difficulties is not new, as shown by the decision during the 1964 Wilson government that the polytechnics should be designated as an alternative system of higher education, one more concerned with practical affairs.

Sourly, the present government has also allowed itself to be captivated by the belief that the whole of higher education is somehow corruptly inefficient. The green paper applauds the recent Jarrett inquiry into the management of six universities but ignores the first of its recommendations, that a prerequisite of efficiency is a coherent policy framework for higher education as a whole. There may be something in the suspicion that British higher education could be operated more economically, although the most obvious diseconomies in the system now are those created by the government's own edicts of recent years. Preoccupied with these problems, the government has taken to behaving as if it wished that higher education would simply disappear. The green paper offers such perfunctory acknowledgement that the system now in being has merit that its readers may be forgiven for thinking that the government has no heart for the struggle to keep the system going.

The crunch will come quite soon. If real resources continue to decline by between 1.5 per cent and 2 per cent a year, whatever slack there is in the system will soon be exhausted. Then the grants committee and the advisory body will have to tell the government that the drain cannot continue within the present framework without striking some whole institutions off the public payroll.

Prudent institutions, universities or polytechnics, will be well advised to get out of the system before that time comes. Reorganizing for independent survival is now the game. The green paper's list of alternative sponsors should (and will) be studied carefully, but there may be others, municipal authorities for example. Only the more depressed among institutions of higher education will ask why should they bother struggling to survive when their present sponsor is so plainly not merely indifferent but ignorant of their role. Some among the others will sense that the claims upon them consist not merely of inputs and outputs ("student demand" and "qualified manpower") but of young people sharing a common conception of what the future might be like; and that knowledge is neither for its own sake nor for the winning of prestige but for a civility that the author and originators of such a banal state document could not comprehend. Groucho Marx is known not to have wanted membership of a club that would let him in. Who, in scholarship and research, dares admit to sponsorship like this? □

Biological manuscripts

Miranda Robertson, Biological Sciences Editor of *Nature*, is now based in the Washington office of *Nature*. Manuscripts offered for publication may be sent, as at present, to either the London or the Washington office; with the benefit of good communications, the speed with which manuscripts are dealt will be independent of the place at which they are received. But authors are asked please in future to send **four copies of manuscripts** intended for publication (one for each office and two for referees).



High-energy physics

UK committee to push for CERN budget cuts

BRITAIN'S spending on high-energy physics is out of balance with its spending on other science, and must be reduced substantially, according to the committee set up last year to consider continued British membership of CERN (the European Organization for Nuclear Research). The committee, chaired by molecular biologist Sir John Kendrew, will report next week to the chairmen of the Advisory Board for the Research Councils (ABRC) and the Science and Engineering Research Council (SERC) that it has been impressed with the excellence of CERN's work, but that the organization's budget should be kept constant beyond 1990.

According to one figure being canvassed in the committee this week, the CERN budget could come down by 20-25 per cent after 1990 without substantially affecting the quality of CERN research, which by then will depend almost entirely on the running of the large electron-positron collider LEP and its extension to "phase II" 100-GeV operation. But whether the final report will actually mention such a figure is uncertain. Committee members are anxious to avoid a conflict with their European partners in CERN, with whom they would like to seek an agreed reduction, and to quote 20-25 per cent might be counterproductive.

Indeed, French officials last week described a cut on such a scale as "likely to kill CERN, given that half their costs are salaries". The West German position is that a 25 per cent cut "could not be done fairly". Nevertheless both countries consider that some cuts are possible. Paris considers that cuts "of a few per cent" would not harm CERN, and Bonn that a 10 per cent by 1990 might be supportable.

Sir John Kendrew and members of his committee met the West German research minister, Heinz Riesenhuber, and the French research minister Hubert Curien, earlier this year, and were encouraged to learn that others might agree that CERN budgets should be trimmed. But the committee did not visit Italy, where the government recently doubled national spending on high-energy and nuclear physics and is in a mood to increase the CERN budget rather than decrease it.

The West Germans told Sir John that the government was more comfortable with the CERN contribution than Britain because certain other projects supported by the research ministry (BMFT) (such as nuclear energy) are decreasing in cost and because, overall, the ministry has been enjoying a roughly constant real budget. He also argued that the expenditure on research and development in most European nations (including Britain) had

decreased as a fraction of gross national product over the past decade, so effective cuts had already taken place. A German committee on big projects under Klaus Pinkau had also set a high priority for CERN and for LEP. Nevertheless Germany is looking for savings.

Mandatory scales of contribution of the eleven member states of CERN, 1985-87:

	Percentage
West Germany	24.90
France	20.23
United Kingdom	16.91
Italy	13.56
The Netherlands	5.39
Spain	4.94
Sweden	4.01
Switzerland	3.87
Belgium	3.61
Denmark	1.98
Ireland	0.60

The CERN budget in 1985 amounts to SF 724.5 million (around £220 million).

Cuts could be found, according to Riesenhuber, in reductions of staff and salaries at CERN. (One complaint is that CERN salaries are so high that it is impossible to attract German scientists back to Germany. There is also, according to Riesenhuber, scope for rationalization of CERN management and in postponing in new investments (such as extending LEP to full performance). A 10 per cent cut by 1990 is "realistic", it is believed in Bonn.

At CERN, however, the prospect of cuts is faced with some horror. CERN director-general Professor Herwig Schopper would like to begin the extension of LEP to 100 GeV per beam as soon as technically

possible, which with rapid advances in superconducting accelerating cavities means spending sooner than expected — in 1989. This would mean a small increase in CERN spending in the new five-year planning period.

There will also be a pensions problem. Schopper says that CERN council decided four years ago to increase staff pensions in line with those available in Switzerland, which will mean increased expenditure on personnel when the main retirement bulge begins in 1990. But CERN has not yet made the investments into the pension fund either to pay for these increases or to pay the basic pension. "Our actuaries tell us there is a 'technical deficit'", says Schopper. CERN is indeed planning a reduction in personnel, and to make more use of outside contractors, but even then "it's unavoidable that there will be an increase in our personnel budget" because of the pension commitments. The only light on the horizon is that Spain will become a full member by 1989, increasing its contribution from the present 5 per cent to 7 per cent of the CERN budget, says Schopper.

For the Kendrew committee, the problem is that even a 10 per cent cut in the CERN contribution would not have provided the increased funding for other sciences that the committee believes necessary. Hence the mooted target of 20-25 per cent, and hence, no doubt, a propensity to be sceptical about Professor Schopper's claims.

Indeed, the feeling in the committee goes even further. First, an attempt should certainly be made first to reach agreement with European partners for a large reduction in the CERN budget. But if that fails, and if the hopes of some members are disappointed that collaboration with the United States will be feasible, the only option will be complete withdrawal from CERN.

Robert Walgate

Caltech at war on star wars

Washington

MARVIN Goldberger, president of California Institute of Technology, has written to Secretary of Defense Caspar Weinberger taking exception to an announcement that Caltech had joined a "consortium" of universities carrying out star wars research. In his letter, Goldberger says that Caltech's only involvement in the \$9 million contract announced by the Innovative Science and Technology (IST) division of the Strategic Defense Initiative organization for research on optical signal processing is a single \$50,000 subcontract to one Caltech scientist from the University of Dayton Research Institute.

A press release put out by the IST office announced the "formation of a consortium of several institutes," including Carnegie Mellon, Stanford and Massachusetts Institute of Technology.

Caltech has yet to approve the contract. Both Caltech and Stanford have also expressed concern about news reports that the work would be classified. But according to Dr James Ionson, who heads the IST office, all the work would be totally free of classification or other restrictions in accordance with Department of Defense policy on basic research that is conducted on campus.

Ionson defended the use of the term "consortium", saying that the scientists involved had worked together on the proposal, which was submitted by the University of Dayton and the University of Alabama at Huntsville. "The important news is that some of the most prestigious individual scientists are working together for a common goal", he said. As to Goldberger, "It's no secret that he's not an advocate of SDI." Stephen Budiansky

UK laboratory animals

New legislation forecast

THE British government has taken a further step towards tighter legislation on animal experiments in a second White Paper (policy document) on *Scientific procedures on living animals*, published last week by the Home Office, the government department responsible (Cmnd 9521, HMSO, £3.30). The document is said to be the result of collaboration between the government, scientists and the "realistic element of the animal welfare movement". The main changes from the earlier proposals (see *Nature* 303, 191; 1983) are: tighter controls on the severity of procedures; modification of the project licensing assessment system; improvements in laboratory animal care; higher penalties for some offences.

Under the new proposals, investigators will need two separate licences, both a personal licence betokening general competence and a project licence for each proposed investigation. Applicants for both kinds of licence must provide the Home Secretary with detailed information about themselves and their proposed work, together with the names of persons willing to act as sponsors. For a personal licence, the sponsor will vouch for the applicant's "competence and integrity". For project licences, the sponsor will give a detailed opinion of the scientific merits of the project and techniques to be used. The white paper says that a field trial has shown that such a system would work in practice. But because of the practical difficulties of assessing all project licence applications in this way, the Home Office now proposed to refer only those projects where the scientific quality of the work is in doubt to an independent assessor.

Criteria for obtaining a licence under the proposed regime will be stricter than at present. The Home Secretary must be persuaded that the work is justifiable, that no satisfactory alternative to the use of animals is available, that the minimum number of animals is used and that the least possible suffering is caused.

Considerable emphasis is placed on the severity of pain, which will be balanced against potential benefit when project licence applications are considered. Such a cost-benefit principle has been widely advocated, in particular by Sir Andrew Huxley, president of the Royal Society, who suggested a graded approach to the justification of experiments on animals in his anniversary address to the Royal Society last year.

The new white paper is an excellent compromise in the debate on animal experimentation, Sir Andrew said this week. The licensing system will make clear who is responsible for a project, and the form of permission for project licences will ensure that granting a licence does not become a "rubber stamp procedure".

In practice, the Home Office will issue personal licences which will, in effect, be a certificate of competence to a particular investigator to pursue a particular project. But project licences will be issued only to applicants "who can satisfy the criterion of permissible purpose". The Home Secretary will impose conditions setting "permissible degrees of severity (of pain) for that project... mild, moderate and substantial". Home Office inspectors will have increased power to order humane killings, "action to be taken at the onset of various signs" such as loss of motor function. The new proposals also require painless killing at the end of a predetermined period, administration of analgesic and constant supervision throughout the procedure.

Before a project licence will be granted, the applicant must agree with the Home Secretary on the likely level of severity of pain and notify an inspector if this forecast is likely to be exceeded. The Home Secretary will decide how the categories of pain are to be defined and applied, and will publish guidelines to inform applicants, licensees and the general public of how the proposals are intended to work.

An animal procedures committee is being set up to advise the government on policy, on applications for research causing "special concern" and on the testing of cosmetics. The committee is chaired by Baroness Warnock and its members include scientists, representatives of organizations that protect animals and representatives of pharmaceutical companies.

The composition of the committee, however, emphasizes the government's commitment to regulated experiments on animals. As Sir Andrew pointed out, the situation cannot arise where experiments can be stopped by a block vote of anti-experimentalists on the committee, which answers the genuine worries of many biological and medical scientists.

Reactions to the white paper are mixed. The Association of British Pharmaceutical Industries accepts the need to update the existing (1876) legislation, but is concerned that there will be "too narrow a definition of the scope of the project licensing provisions". The Humane Research Trust and the British Veterinary Association welcome the proposals in the main. The British Union for the Abolition of Vivisection calls them a "confidence trick" and a "vivisectionists' charter".

The number of experiments on animals in the United Kingdom has fallen from 5.6 million in 1971 to 3.6 million in 1983. In 1985 the Humane Research Trust will give grants of £100,000 to nineteen investigators using alternative experimental procedures that do not animals, and the pharmaceutical industries will spend £10-12 million on development of non-animal techniques in drug testing.

Maxine Clarke

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US laboratory animals

NIH watchdog committees

Washington

LABORATORY animal research at publicly supported institutions in the United States will from November this year have to be overseen by special "animal use" committees that will include at least one lay representative, according to new guidelines issued by the Department of Health and Human Services. The guidelines charge the animal use committees with responsibility for reviewing research programmes and inspecting laboratories and animal houses at least annually. Research that does not meet the committees' standards can be stopped.

The requirement that each committee should include at least one lay member is welcomed by animal welfare campaigners, but some had hoped for a representative concerned specifically with welfare. Welfare champions are unhappy that the committees are not required to review in detail research protocols for investigations in which non-trivial pain may be caused.

Christine Stephens, president of the Animal Welfare Institute, says that this omission is a "profound disappointment". Earlier proposals had required special approval by the committee for non-routine or harmful invasive procedures, procedures involving prolonged restraint, diseases maintained for long periods or non-standard methods of euthanasia. The new proposals leave it to committee members to decide if special attention is warranted. Ms Stephens is also disturbed that institutions can apparently apply for a retroactive waiver that would release them from their responsibilities under the new guidelines.

The Association of American Universities, in contrast, is relieved that lay members will not be required to review research protocols, a requirement that the association considers "unacceptable".

The guidelines do not have the force of law, although an institution could in theory lose its financial support if deficiencies went uncorrected for long enough. Critics point out, however, that such an occurrence is almost unknown. The National Institutes of Health are still investigating allegations of mistreatment of baboons used in head injury experiments at the University of Pennsylvania; despite the widespread availability of a video tape showing apparent mistreatment, which was last week being shown to members of Congress, the research is still being supported. A bill introduced in the last Congress by Senator Robert Dole and Representative George Brown would have given statutory force to animal use committees: a new version of the bill is likely to be introduced in Congress within the next few weeks.

Tim Beardsley

DNA probes

Type culture a source of gain

Washington

THE role of the American Type Culture Collection (ATCC) as a repository of materials for basic biological research is being threatened, researchers say, by commercial exploitation of cell lines deposited there. At least one molecular biologist has refused to send any more of his samples to the collection after a commercial company obtained an oncogene he had deposited and, without his knowledge, began marketing to researchers DNA probes derived from it.

ATCC, a non-profit organization, makes cell lines and other materials in its collection available to any qualified researcher who requests them, charging a nominal fee to cover its costs. Although primarily a service to basic researchers, the collection has played an increasingly important role in facilitating the patenting of recombinant DNA products and processes.

As a general rule, the Patent Office requires applicants to deposit novel cell lines or plasmids in a collection such as ATCC to satisfy the public disclosure requirements of patents — the traditional written descriptions are often insufficient to allow someone else to reproduce the invention, a fundamental requirement of patent law. In order to satisfy the legal requirements, no restrictions on the distribution of deposited materials can be imposed.

But that totally unrestricted distribution is upsetting some basic researchers. Tom Curran, a molecular biologist at Hoffmann-La Roche, recently deposited a cell line containing the *fos* oncogene, which he had isolated when he was a graduate student at the Imperial Cancer Research Fund Laboratories in London, in collaboration with Inder Verma of the Salk Institute.

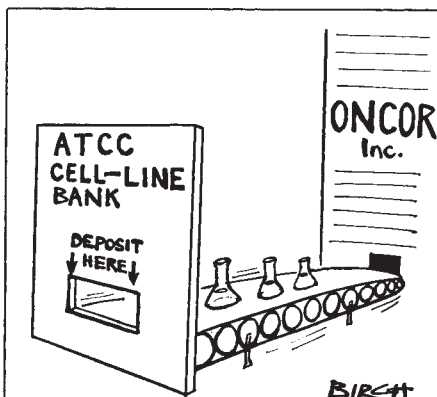
Curran says he was subsequently "shocked and distressed" to discover an advertisement in *Nature* from Oncor Inc. for DNA probes for a variety of oncogenes, including *fos*. Curran says Oncor did not even inform him that it had obtained the sample from ATCC or that it was planning to market his product. He says his primary objection is that Oncor is making a profit by selling to basic researchers a product developed by basic research in which the company had no part. Curran says he deposited the oncogene with ATCC so that it would be "freely available to the scientific community".

Some research groups, apparently concerned about the uncontrolled commercial exploitation of cell lines placed in the public domain through ATCC or otherwise, have taken to distributing samples themselves to other basic researchers and requiring a signed agreement that those receiving them will not pass them on to a third party or exploit them commercially without permission. Although there is some dispute over the enforceability of such agreements at law, in

at least one well-publicized case, that of David Golde's KG-1 cell line, the originators of the cell line won a sizeable out-of-court settlement from a company that had obtained and was using the material without their permission (see *Nature* 301, 6; 1983).

Bobbie Brandon of ATCC says that the collection may have to reconsider its policies on free distribution, but that it would not want to be in the position to enforce restrictions against transfers or commercial development. And, although she sympathizes with Curran's complaints, she says Curran had signed a statement agreeing that ATCC could make the oncogene available to anyone requesting it. Brandon says that ATCC distributes about 1,000 oncogene samples each year at a cost of \$64 to commercial organizations and \$44 to non-profit groups.

Steven Turner, founder and chief executive officer of Oncor, says he sees nothing wrong with the service that his company is providing and that his com-



pany's contribution is by no means trivial, as Curran suggests. "We're not just taking the ATCC strain and dropping it into tin cans and selling it", he says. Turner adds that the customers for the products are not basic molecular biologists, but rather clinical researchers who do not have the means to develop the probes themselves from the material available from ATCC. And he says that strains deposited in ATCC are in fact not very attractive for commercial development because they are in the public domain: "I have no protection with what I do with that strain".

Turner, who was the founder of Bethesda Research Laboratories before founding Oncor two years ago, says, "I long ago got used to the hypocrisy of academics saying that their activities are on a higher moral plane than business". While academic researchers refuse to sell their cell lines, they nevertheless demand a *quid pro quo*, such as demanding that the recipients "stick their names on your papers for the next five years". "I've never seen exploitation as I've seen it performed in academic labs."

Stephen Budiansky

Sakharov

Birthday award from US centre

Washington

THE Andrei Sakharov Institute in Washington, founded five years ago to honour the Soviet academician and human rights campaigner, this year added a new feature to the now-traditional Sakharov birthday celebrations — an annual prize for science. The first recipient is Dr Aleksandr Esenin-Volpin, a mathematical logician, for many years a human rights campaigner and a victim of psychiatric repression in the Soviet Union, before he was "persuaded" to emigrate in 1972.

The prize this year is modest, a mere \$5,000, and the decision to establish it was taken only two weeks before the event. Vladimir Bukovskii, himself a former human rights campaigner and psychiatric victim, said in announcing the award that the funds were largely collected from Russian emigres in the United States, many of whom are not well-off. But in view of the number and distinction of the universities and institutions from which the "advisory board" of the Sakharov Institute is drawn, and the recent introduction of a congressional bill to grant it a federal charter, there is every hope that a more substantial sum may be available next year.

The prize is not the institute's first educational venture. In addition to its Center for Democracy, which aims at propagating Sakharov's ideas on peace and democracy and publishing in English the works of authors unable to express themselves freely in their own countries, it also maintains an education and research section, which has been adopted as the Washington Campus of Long Island University. This has two major projects in hand, the improvement of the quantity of mathematics and science education in the United States (including the establishment of a National School for Science and Mathematics for Gifted Students) and the upgrading of the quality of Soviet and East European studies.

The announcement of the new prize added an optimistic note to last week's Sakharov "birthday" conference (held a few days before the actual date, 21 May), which otherwise concentrated on current progress (or the lack of it) in international acceptance of Sakharov's views on peace, arms control and human rights.

Inevitably, the issue was raised of plans to renew contacts between the US National Academy of Sciences and the Academy of Sciences of the USSR (see *Nature* 16 May, p.169). Since the National Academy broke off relations with the Soviet Academy in protest at Sakharov's internal exile to Gor'kii, it is not surprising that those gathered to honour him felt that there should be no new approach to the Soviets while Sakharov and his wife remain in exile.

Vera Rich

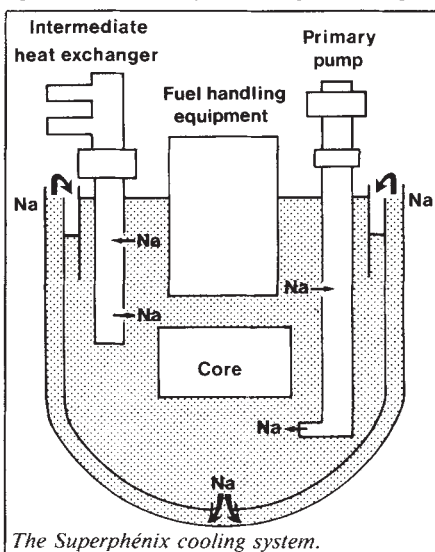
Fast reactors

Superphénix vibrations solved

VIBRATION problems in the primary sodium cooling circuit of the Superphénix European commercial-scale fast breeder reactor "have been solved or nearly so", and will not delay the fuelling of the reactor as had been feared, senior officials at the French Commissariat à l'Energie Atomique (CEA) said last week.

Thus the 1-200 MW(e) Superphénix, which has been built at Creys-Malville on the Rhône by a consortium in which the French and Italians are the major shareholders, with West Germany third, will still be loaded with plutonium at the beginning of September and produce its first fast neutrons soon after, according to CEA.

The problem now considered all but solved has been with an inner jacket of the main reactor vessel, which is little more than a tank full of liquid sodium, topped up with an inert argon atmosphere. As part



of the cooling circuit, a thin film of sodium passes upwards from the bottom of the main vessel around the outside of the inner jacket, before spilling inwards over the lip of the inner jacket into the core of the reactor. In the first live tests of the cooling system full of heated sodium, the jacket was vibrating with an amplitude of some 12 mm in a diameter of 20 m.

It seems, however, that CEA will now go ahead with the fuel loading without modifying the reactor. Engineers at Creys-Malville have found that a slight adjustment to the sodium flow rate will stop the vibration. The key factor, according to CEA, was the height through which the sodium fell when cascading over the lip of the inner jacket. Initially 100-150 cm, this "sodium fall" has been reduced to 30-60 cm by increasing the volume of sodium in the reactor (by 200 tons) and slightly increasing the flow rate between the inner and outer jackets.

Meanwhile discussions continue in Europe over, first, where to site a planned second fast-breeder reactor, with West

Germany and France the main contenders, and second, where to place a single fast fuel reprocessing plant, with the main contenders being France and the United Kingdom. CEA sources believe that a

Belgian research

Autonomy sacrificed to regions

Brussels

REGIONAL tensions in Belgium are eroding the autonomy previously enjoyed by the quasi-governmental institute for the encouragement of scientific research in industry and agriculture (IRSIA). Hitherto, the institute has been free to select areas and projects for applied industrial research, but now its freedom will be further limited by the latest separate decisions of the fiercely competitive Flemish and Walloon regional ministers to increase their influence on its affairs.

Last month, the president of the Flemish regional executive, Gaston Geens, announced that IRSIA would from now on have to submit projects received from Flemish companies for approval to a commission directly under the control of the minister of economics before funds could be granted. It is also required that one-third of the money available should be specifically set aside for small and medium-sized companies, that a certain amount should also be earmarked for projects specified by the regional executive and that all Flemish companies would have to pay back subsidies obtained from IRSIA when the products of research reach the commercialization stage.

Until now IRSIA has always believed in a system of non-repayable subsidies on the basis of a 53 per cent average participation for regular projects and 80 per cent for first ventures, with success and quality governing future eligibility. The old system remains in force in the Walloon and Brussels regions.

The new Flemish system, it is feared, could have the double effect of wilful under-estimation of results by companies to avoid repayment of the loan and could even restrict technological innovation.

Since 1983, when research was defined in terms of the 1980 Belgian regionalization laws, IRSIA has become used to administering separate coffers (the proportional distribution system was revised in August 1984 to try to give each region its fair share). The major industrial sectors of Belgium — glass, textiles, coal, steel and construction — have not been affected by regionalization, and nor have large companies and collective research centres, but IRSIA previously supported small companies not only with money but also with information on research activities elsewhere. Regional separation has meant that

decision on the site and detailed design of a second breeder is necessary by 1987, and that only France could be ready by that date. The potential purchasers, the French national electricity utility Electricité de France, however, which will probably have a large surplus of electricity by the end of the decade, is not thought to be quite so keen.

Robert Walgate

research activities are suffering both restrictions and overlap. It has also put IRSIA in a very delicate position: it receives applications and oversees projects from all regions but it has to keep information about research developments in each completely separate.

As well as making extra administrative work for IRSIA, the latest decisions also risk fragmenting the applied research effort in Belgium as a whole. This seems ironic at a time when the European Commission, in the Belgian capital, Brussels, is urging the ten member states of the European Community to make easier the mobility of workers, the exchange of information and cooperation on research. Anna Lubinska

Fetal diagnosis trial

CHORION villus sampling, a new technique by which genetic fetal abnormalities can be detected as early as eight weeks of pregnancy, is to be evaluated in a three-year clinical trial run by the British Medical Research Council (MRC). Amniocentesis, the usual method for prenatal diagnosis of genetic disorders, has the disadvantages of being performed later in pregnancy (about 15 weeks) and, because cells have to be grown in the laboratory, the results are generally not available for a further four weeks.

In chorion villus sampling, a sample of placenta containing fetal tissue is removed, allowing immediate diagnosis and much earlier termination. But the technique may be less safe. Preliminary indications (using the 3-400 tests performed so far in the United Kingdom) suggest that the spontaneous abortion rate is 2-5 per cent, compared with 0.5-1.5 per cent in amniocentesis. The randomized trial will include 4,000 women in the United Kingdom and is based on the informed consent of women who elect to join the scheme. Between 10 and 20 UK and European centres will take part; the study will be coordinated with Canadian and US trials. MRC will also follow up the children born after the prenatal trial to assess for any long-term effects of the technique.

Diseases such as Down's syndrome, anaemias, haemophilias and phenylketonuria can be identified by prenatal sampling; soon muscular dystrophies and cystic fibrosis will be detectable as genetic probes become available.

Maxine Clarke

UK higher education

Help business and survive

THE British government's long-promised Green Paper (discussion document) on higher education, published earlier this week (Cmnd 9524, HMSO, £5.80), does nothing to suggest that the pressure on university and polytechnic budgets will be relieved. Indeed, the government says that planning for the impending fall in student numbers, expected on demographic grounds in the 1990s, "will need to begin shortly". The document says that these changes will require "not only rationalization within institutions but the amalgamation or closure of whole institutions".

The document says that there is a need for "means of assessing, on educational and economic grounds", where capacity should be reduced, promises that the chief advisory bodies (the University Grants Committee, UGC, and the National Advisory Body, NAB, which represents the polytechnics), will "have a key role" and adds that "the government will be fully engaged". The government notes the pleas of both bodies as well as of the Jarrett committee on university administration (see *Nature* 4 April, p.392) that institutions should be given longer notice of the public funds at their disposal, but says that its three-year rolling forecasts are the most it can provide, and that higher education institutions should be able to accommodate "change and some inevitable uncertainty".

To most academics, the green paper will be a disappointment, not for what it says or neglects to say but for its tone. The keynote is the statement in the introductory section that "the government believes it is vital for our higher education to contribute more effectively to the improvement of the performance of the economy". Referring to its own initiatives to improve the quality of science teaching in schools, the government says that there "would be no point in continuing to provide more places in science and technology in higher education" unless there are enough able students to fill them, urges that universities and polytechnics should plan for flexibility in the face of change and demands that "higher education establishments" should abandon "anti-business snobbery" and, instead, should foster entrepreneurship, should "go out and develop" links with industry and commerce and should form strong connections with local communities.

With qualifications, the green paper accepts the redefinition by UGC and NAB of the principle that suitably qualified students should have access to higher education (the "Robbins principle") by agreeing that "courses of higher education should be available to all those who can benefit from them and who wish to do so". The qualification is that "so long as taxpayers substantially finance higher education . . . the benefit has to be sufficient to justify the cost". The "test of

ability to benefit" should be applied as stringently to those with formal qualifications as to those without.

Inevitably, the green paper rehearses the government's position on the future demand by students for places in higher education, but promises a more considered estimate of demand after a survey now being carried out among young people. Meanwhile, it says, the funds now promised should suffice to allow universities and polytechnics to meet the demands on them in the three years ahead. The document says that UGC and NAB have been asked to recommend how students should be distributed between the universities and the polytechnics, but asks that students should not be admitted to universities if they are more likely to benefit "in terms of personal development and future employment prospects" from a course at a polytechnic.

On research, the green paper rejects the argument of UGC that "research stimulates the teaching of able students" with the statement that "there is no evidence that all academic staff must engage in research". It applauds UGC's plan to distribute research support selectively, hopes that a start will be made in 1986-87 and notes that one consequence may be that "some departments or even whole universities will lose research funding (*sic*) from the UGC". The green paper says that universities will still be free to seek the funds from private sources.

With more enthusiasm, the green paper argues the benefits that would accrue to

academics and to "business" from a closer relationship between them, and urges academic institutions to take account of industrial consultancies when assessing candidates for promotion, to encourage academics to "engage in business activity", if necessary on part-time academic contracts, to strike "reasonable deals" with staff over the income earned from innovations and to operate industrial liaison services. (Cautiously, the green paper warns academic institutions of their potential insurance liabilities in these activities.) In the same spirit, the government says it has asked UGC that success in industrial collaboration should be given "due recognition".

The green paper also says the government is not convinced that two-year degree courses are feasible, but would like to see them tried, and that it is dismayed at the growth of the numbers of students now following four-year courses. The paper also says that the government is planning to construct measures of performance for higher education institutions, and that it wishes there to be a uniform system of validation of university courses. On academic tenure, the green paper says that the academic freedom of those who lack this privilege "is not visibly under threat". There is to be an inquiry into the role of UGC.

One of the recurrent themes of the green paper is the right of free speech within universities, and the government promises a reconsideration of the degree to which students' unions should be financed from public funds. Meanwhile, it asks that universities and polytechnics should do what they can to prevent students from suppressing unpopular views. □

Interferon

Agreement on marketing

Washington

HOFFMANN-LaRoche Inc. and Schering-Plough corporation have reached a truce in their battle over marketing rights for human leukocyte (alpha) interferon (see *Nature* 21 March, p.207). Under the terms of agreements reached last week, Schering-Plough will pay royalties to Roche if Schering-Plough's product is sold in the US market, while in Europe each will be free to sell its own product without infringing the other's patent rights. Terms for Japan are expected to be similar to those covering the United States.

Schering-Plough's interferon (trade-marked Intron) is produced under licence from Biogen NV of Cambridge, Massachusetts and Geneva, Switzerland. Biogen holds a European product patent for alpha interferon produced by recombinant techniques and expects to receive a similar patent in the United States. Roche, however, holds a very broad US patent that covers all highly pure human leukocyte interferons, and the new agreements

recognize that Schering-Plough's product falls within the terms of the Roche patent.

The agreements mean that recombinant interferons will be commercially available while the patent claims are sorted out. Roche has challenged Biogen's European product patent and that challenge will be pursued despite the agreement that both can market their products; the outcome would be important if either company decided voluntarily to withdraw from the market, for example. In the United States, where both Intron and Roche's "Roferon-A" alpha interferon are in clinical trials, the final outcome of the dispute will hang on the scope of the expected Biogen patent for recombinant alpha interferon. The agreements refer only to a specific type of alpha interferon, however; if other variants prove to have valuable properties, there could be more battles to come. In addition, both beta and gamma interferons are in clinical trials and marketing rights for them are likely to be keenly contested.

Tim Beardslev

UK research

Despairing demand for more

THE Oliver Twist of British civil science, the Advisory Board for the Research Councils (ABRC), has this year asked for more in even stronger language. The preface to the annual claim for research funds, agreed by the board at its meeting at the end of April and now on its way to Sir Keith Joseph, the Secretary of State for Education and Science, says that "Parliament, the government and the country as a whole" are "complacent" about the present condition of the dual support system, the means by which support for university research is channelled through the research council and the University Grants Committee.

ABRC says that this complacency is born of a misconception of the function of the research councils and of basic research as such. The board thinks it necessary to point out that it is not true to say that the research councils support only research for its own sake and that academic research is remote from the real world.

As in previous annual applications for funds, ABRC acknowledges that there are limits to what can be afforded for basic research. But it insists that the government which ultimately foots the bill cannot expect that new technologies will emerge unless there is basic science from which they can spring. The board's application also argues that the pace of scientific advance is now such that copying other innovative technology is no longer a recipe for industrial success.

Part of the board's difficulty, this year as previously, is that the government appears on the face of things to have kept the Prime Minister's promise in 1980 that the research budget would be "protected". Using the retail price index as a measure of inflation, the science budget has in fact increased by 6.4 per cent since 1981-82. Even allowing for the decline of the research councils' other income, mostly by way of research commissions from government departments, amounting to roughly 7 per cent on a sixth of the total science budget during the same period, the total income of the research councils has increased by roughly four per cent.

The board says that this simple arithmetic is misleading partly because the retail price index is not a good measure of the increased cost of research. Rather, the board calculates that the real volume of research supported with the funds within its purview has declined by 5 per cent over four years. Matters have been made worse, the argument continues, by the 8 per cent reduction (in real terms) of the budget of the University Grants Committee.

The case is sustained by a harrowing account of the changes which have been brought about within the research council system in the past few years. The total staff of the five research councils has declined

by a fifth, by 2,000. But in general, ABRC says, the councils have found that the rate at which they are able to fire or redeploy staff is itself constrained by their shrinking budgets.

The consequences of this state of affairs are catalogued in detail. Thus, the submission to the government is believed to say, the Science and Engineering Research Council is no longer able to make full use of new facilities because it cannot afford the research equipment, is having to pass up opportunities for international collaboration for lack of funds, is unable to meet its commitments (to other government agencies) to encourage information technology and cannot give adequate support to several named areas of research of vital industrial promise.

Other lost opportunities cited by ABRC include the Medical Research Council's incapacity to provide more than half the equipment needed for a new programme of

research in protein engineering at the Laboratory of Molecular Laboratory at Cambridge, the Natural Environment Research Council's incapacity to participate in the new ocean drilling programme and the incapacity of the Agricultural and Food Research Council to afford more than a third of the cost of replacing outdated research equipment.

By comparison with previous submissions, the latest request for more research money is terse, almost despairing, as if it had been judged imprudent that an unwilling government should be confused with a plethora of facts. ABRC will protest separately about the way in which the budget of the Agricultural and Food Research Council has been cut by £5 million this year and by twice as much in succeeding years because of a Treasury edict. Meanwhile, ABRC's modest request of government is £15 million for the year beginning next April, twice as much in the succeeding year and £40 million in 1988-89.

On past form, ABRC's submission and the response to it will be published in the autumn. □

UK neuroscience

New lab, old unit in new role

BRITISH neuroscience was given a much-needed fillip last Friday with the inauguration of the new £25-million Merck Sharp & Dohme Neuroscience Research Centre near Harlow, Essex, which is dedicated to investigating chemical transmission processes in the brain. The hope is that one outcome will be the development of new drugs for treating neurological and mental disorders. The centre is at present paying particular attention to senile dementia (Alzheimer's disease) and inhibitory pathways.

The centre is directed by Dr L.L. Iversen, formerly head of the Medical Research Council (MRC) Neurochemical Pharmacology Unit at Cambridge. It will eventually employ about 160 professionals and 50-60 support staff, making it one of the largest brain research centres in the world. It comprises departments of medicinal chemistry, biochemistry and pharmacology, including neurophysiology and animal behaviour, but project teams will be interdisciplinary, involving staff from various departments.

Iversen emphasizes the goal of designing drugs based on a knowledge of the structure and function of the membrane receptor sites for the appropriate transmitter chemicals, instead of the empirical synthesis of chemicals which are then screened for their effects on behaviour. The centre also includes a small clinical research unit to undertake preliminary trials of new drugs. There will be collaboration with universities, particularly under research council schemes for joint direction of research studentships, and with hospitals.

According to Merck Sharp & Dohme.

the centre has been established in Britain because of the "pathfinding leadership of British scientists in brain research". Its existence should provide much-needed encouragement to the younger generation of British neuroscientists. The grim job prospects in academic research are resulting in fewer good students going into neurosciences and many that do are leaving Britain after their training. The centre has already attracted several younger scientists back from the United States.

Meanwhile, the Neurochemical Pharmacology Unit at Cambridge, threatened with closure a year ago, has been reborn. After a controversial two-year search to replace Dr Iversen as head of the unit (see *Nature* 309, 486; 1984), MRC has appointed Professor E. Barnard of the Biochemistry Department, Imperial College, London, as the new director of the unit, now to be known as the Molecular Neurobiology Unit, from 1 October. In the same building as the MRC's Laboratory of Molecular Biology in Cambridge, Barnard's laboratory is intended as the focus in Britain of the use of molecular genetics techniques to study neurotransmitters.

The new unit will have two main lines of research — the development of animal models for the study of muscular dystrophy and motor neurone disease, and the cloning of brain receptors and their genes, in particular in nicotinic acetylcholine receptor and receptors for neuropeptides such as enkephalins and substance P. At full strength the unit will comprise five senior scientists and about 35 visitors, post-doctoral fellows, students and technicians.

Jennifer Altman

More on nuclear winter

SIR — The intrinsic ambiguity of numerical simulations of atmosphere processes guarantees a protracted controversy about the phenomenon popularly called "nuclear winter", as for example described by Turco *et al*¹.

There are, however, ways of injecting large masses of soot into the high atmosphere that are phenomenologically more explicit than those now described. It is necessary only to tell a systems programmer where one guesses the source of carbon should be and to ask him or her please to adjust the computer model accordingly. Consider targeting a coalfield.

The integration of biomass over geological time has endowed millions of square kilometres of the Earth's surface with fossil fuel loadings in the range from 100 to 10,000 g cm⁻² (ref. 2), compared with the 1 to 50 g cm⁻² that constitutes the carbon budget of the rural and urban fires in ref. 1. An unknown fraction of this ubiquitous coal, lignite, oil shale and peat is either exposed or lies beneath a thin veneer of overburden³, and is at risk of interaction with energetic phenomena, including thermonuclear surface bursts and cataclysmic volcanism.

Warheads with yields in excess of 0.5 megatons detonated in such carbonaceous terrain can promptly transform the ground beneath into a mixture of carbonaceous plasma and particulates launched towards the stratosphere by direct entrainment in the rising fireballs. Efficiencies of roughly 10 to 100 megatons of soot per gigaton exploded seem likely (J. Nuckolls and L. Wood, personal communication). It should be noted that NATO (North Atlantic Treaty Organisation) and Warsaw Pact missile fields and fossil fuel fields are partially co-extensive, as are the loci of cataclysmic calderas and the massive (trillion ton) hydrocarbon resources that occupy ≥ 10 per cent by area of the western United States.

Every few hundred thousand years, that region is host to a randomly located eruption of 250 to 2,500 cubic kilometres of incandescent tephra. Dr Turco has expressed his willingness to accept the largest of these events as analogues in their climatic impact to the dust baseline of ref. 1. In recent geological history, these eruptions have eviscerated about 5 per cent of the area in question. The worst-case scenarios arising from the interaction of caldera volcanism and fossil fuels involve both carbon and energy budgets that dwarf its inputs of nuclear winter. What were the consequences — local or global, mild or catastrophic?

A survey of the extent of their geological events and of their palaeoecological consequences might serve to illuminate the extent to which the nuclear winter model is a valid predictor of the fate of the Earth. Since a rough calculation yields a mean

time of rather less than 10 million years between events capable of producing catastrophic natural carbon injections, the fact that we are not extinct may be germane to the present controversy.

It remains to be seen whether the neologism "nuclear winter" will ever qualify as what philosophers of language call a "rigid designator in the real set", two examples of which are "Carbon County, Wyoming" and "The Yellowstone Caldera". I do not mean to consign "the nuclear winter" to the null set, exemplified by "the canals of Mars" and "the unicorn", but merely wish to point out that a fossil rhinoceros in the hand is more amenable to analysis than two unicorns in the software, and that a diligent search of the fossil record has turned up many a rough beast in the past.

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Nullius in verba

SIR — In your reference to the motto of the Royal Society (*Nature* 2 May, p.9) you make two mistakes. First you misquote the motto itself, and then you give a totally false translation.

The motto is *Nullius in verba*, the word *nullius* being the genitive whereas you give *nullus*, the nominative.

Admittedly, the meaning of the motto is obscure until one knows the rest of the line of Horace (*Epistolae*, I, i, line 14) from which the words are taken. This reads: *Nullius addictus jurare in verba magistri*, which may be translated as "Not committed to swearing by the words of any master". Here Horace declares to his patron Maecenas his own attitude to authority, and it is this rejection of authority as a source of truth that the Royal Society expresses in its motto. Your translation, "nothing in words", is wide of the mark.

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Mass extinctions

SIR — Coming when it did, Michael Benton's article in News and Views (*Nature* 11 April, p.496) must have appeared like manna from heaven to the hundreds of geology and zoology undergraduates throughout the country, and presumably at his institution too, preparing for their final examinations this summer.

It is therefore important to note that Benton chose not to include the single most parsimonious hypothesis underlying the phenomenon of mass extinctions, namely that extinction events are the result of any number of external but unrelated causes which, as a result of simple probability, appear to occur with regularity.

Whether he likes it or not, this single hypothesis, concerned as it is with historic events, will perpetually thwart any attempts to falsify it. The demonstration of cyclicity paralleling these events does not establish a cause/effect relationship. The isolation of a likely cause in one or two cases cannot be extrapolated to the whole. Similarly, the prediction of the cause of the next is not going to help the debate now.

We are left with five explanations for mass extinction cycles. Arguments for and against each view, though futile, will continue. More detailed analyses of the fossil data, and refinements in dating events in the geological past, will not help us to decide which view is correct, though they may enable us to say which is most likely.

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Future of RGO

SIR — Philip Campbell commented in *Nature* (28 March, p.303) that the staffs of the Royal Greenwich Observatory (RGO) and the Royal Observatory Edinburgh would greet with weary shrugs of resignation the news that the Science and Engineering Research Council (SERC) is to set up yet another committee to decide their future.

Dr Campbell is right in the sense that we have been subjected to so many of these exercises, this will be the fourth since 1983, that much time which should have been spent on observatory projects has been dissipated in justifying our existence.

The staff of this observatory are certainly not, however, facing SERC's latest investigation in a spirit of resignation. On the contrary, we believe that what we have achieved and are continuing to achieve in spite of under-funding and uncertainty is proof that RGO is a viable and efficient base for the support of astronomical research for the United Kingdom and its international partners.

Moreover, the scientific damage likely to be caused by any major disruptions at this stage when we are completing the new observatory on La Palma is too serious to contemplate with anything approaching resignation. The staff at RGO will be entering into discussions on the situation with vigour and determination.

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Trailing AIDS in Central Africa

A meeting in Senegal enabled AIDS researchers to learn more of the possible African origin of the virus that causes AIDS and virus-associated cancers in Africa.

BECAUSE of growing evidence that the virus that causes acquired immune deficiency syndrome (AIDS) originated in Central Africa, AIDS researchers are turning to their atlases. Earlier this month, on 8-10 May, some landed there for the first time to attend a meeting arranged by the French Association pour la Recherche sur le Cancer at the faculty of medicine in Dakar, Senegal, on virus-associated cancers in Africa. What they were forcibly reminded of is that in African terms, AIDS is an insignificant problem, but that most common forms of cancer in Africa are caused in part by viruses.

At times the question of the origin of AIDS has seemed to be no more than a matter of geographical buck-passing — first between the United States and Haiti and then to and within Africa — but the issue is much more serious. At its worst, the argument is that the virus (HTLV-III or LAV) has only recently emerged in virulent form, has been able to do so as a result of changing socio-economic factors in Africa and may be followed by equivalent conversions to virulence of other relatively harmless viruses. More optimistically, the question becomes: can one learn anything from the origin of the virus that would help to contain the disease?

Geographically, the finger of suspicion points at Central Africa, possibly Zaire, as the location from which the virus has spread. The data, however, are sketchy and dependent on what historical and contemporary sera samples are available for analysis, and to whom. From one of the most extensive surveys so far, G. de Thé of the CNRS, Lyon, provisionally concluded that the virus emerged in Kenya only after 1970, that there are still very puzzling differences between adjacent countries, and that there may be evidence of change of viral form in some comparative data.

The evidence of change comes from the comparison of two tests, one of which (an enzyme-linked immunosorbent assay) is less sensitive to change than the other ("Western blotting" of proteins). That the current virus is very prone to change is clear. The sequences of viruses that came directly or indirectly from patients in New York in 1982-83 differ by only a few per cent, whereas the sequences of a virus from a Haitian taken in the same period and one from a resident of San Francisco isolated in 1984 show considerable differences. Most variation is in the mid-portion of the gene that encodes the envelope protein of

the virus. Up to 25 per cent variation is recorded there, according to W. Haseltine of Harvard Medical School.

By definition, however, all of these isolates and the other hundred or so whose variation is fully documented in only a few cases, are from AIDS patients. All, therefore, have caused immunodeficiency, and no correlation has emerged between variation in viral structure and the precise manifestations of the immunodeficiency syndromes. The pressing question is whether such a correlation can be found and, in particular, whether the African population will yield variants that are less prone to cause AIDS and from which the "American" variants are descended. In other words, do viral differences account for the fact that AIDS in Central Africa seems to be equally distributed between the sexes and transmitted by heterosexual activities rather than largely by homosexual practice and blood or its products? Or are these differences only a temporary reflection of the initial entry of the virus into the industrialized nations through the homosexual community? The signs are more that the latter is the case.

Now the focus of attention has shifted to the African green monkey. Most of some recently collected specimens from the wild in Zaire contain serum proteins characteristic of HTLV-III/LAV and yet are healthy, according to M. Essex from Harvard School of Public Health. A comparative study of the monkey and human virus together with that which caused an outbreak of AIDS-like disease in a captive colony of Macaque monkeys in the New England Primate Center may help to clarify the origin and virulence of HTLV-III/LAV and suggest approaches to vaccination or therapy.

A separate reason for delving deeply into variants is to discover whether sequence changes are responsible for some of the human pathologies other than AIDS with which HTLV-III/LAV is coming to be associated. R. Gallo from the National Cancer Institute in Bethesda, Maryland, pointed in particular to an association with brain abnormalities in children without immune deficiency; evidence exists for the presence of replicating virus in the human brain, particularly in glial cells. Such evidence tends to strengthen the claims of both Gallo and S. Wain-Hobson, of the Institut Pasteur in Paris, that HTLV-III/LAV is related to the lentiviruses, of which the prototype is visna virus, which

causes a slowly progressive demyelinating disease of the central nervous system of sheep. Gallo also tentatively suggested a new pathological involvement of the virus that would dispense with the first word of idiopathic thrombocytopenic purpura.

While AIDS is a growing concern in the industrialized nations, African priorities lie elsewhere. In Senegal, for example, either there is no AIDS or it has not yet been recognized, whereas many die from a liver cancer caused in part by hepatitis B virus. In Africa generally this, and cervical cancer are major types of cancer. A third virus-associated cancer in Africa is Burkitt's lymphoma, an infrequent disease but responsible for many childhood tumours and causally linked to the Epstein-Barr virus.

None of these viruses causes cancer without contributory factors, of which immunodeficiency is the most interesting to AIDS researchers. For Burkitt's lymphoma, immunodeficiency — in the form of lost of T-lymphocyte control of Epstein-Barr virus infected B cells — is induced by malaria, as shown by H. Whittle of the MRC Laboratories, The Gambia. And a mini-epidemic of Burkitt's lymphoma in US AIDS patients suggests that any form of immunosuppression is liable to activate the latent virus.

Hopes, however thin, are pinned on the development of a vaccine against HTLV-III/LAV. For the hepatitis B virus, vaccines are already in trial but for the Epstein-Barr virus the first glimpse of a vaccine was provided in Senegal by M.A. Epstein of the University of Bristol. The vaccine is composed of one of the major proteins of the virus and protects cotton-top tamarins against cancers induced by the virus. The tamarin disease does not altogether resemble the human disease and the frequency of Burkitt's lymphoma may never warrant mass vaccination in terms of cost. In Asian countries where the virus is a part cause of nasopharyngeal cancer, vaccination may be more realistic. Epstein foresees an initial trial of the vaccine against mononucleosis, the relatively mild result of infection by the virus after childhood.

The AIDS researchers who dropped in on Senegal need African collaborators in order, at the least, to carry out extensive trans-African surveys of AIDS, its virus and its origins. The Africans leaving Senegal knew at least that they had increased the awareness of the diseases and priorities in Africa among those who could provide help.

Peter Newmark

Isotope geochemistry

Contamination or source-region heterogeneity?

from Alex Halliday

OVER the past thirty years considerable effort has been invested in using the isotope compositions of certain elements (principally Sr, Pb, Nd and O) to reveal information about the chemistry and history of the source regions of magmas. There are two causes of variation in isotope ratios — radioactive decay (for example, the production of ^{87}Sr from ^{87}Rb) and mass-dependent isotope fractionation during reactions. In the first case, the isotope compositions tell us about the chemical history (that is, the age and parent/daughter elemental ratios) of the source. In the second case we can, in addition, learn about some specific processes that operated in the magma itself. Such data have been fundamental in solving major petrological problems such as the origin of granites and basalts. This has led to a relatively detailed knowledge of the chemical structure and evolution of the Earth's mantle and of the growth of the continental crust.

But the case is not always as clear cut. There is still debate over the interpretation of the isotopic characteristics of rocks believed to have formed in the mantle but to have risen through the continental crust. The argument centres on the relative roles played by contamination by the continental crust and inheritance of features from the sub-continental mantle. The problem is fundamental to the origin of igneous rocks and is made worse by the range and complexity of potential contamination mechanisms, for example, by fluids or partial melts, which produce non-diagnostic effects. One school of thought discounts crustal contamination on the basis of banal assumptions about the probable effects, whereas those from the other school simply ignore the possibility that anomalous mantle is important. Attempts to resolve these views have often resorted to lengthy convoluted arguments.

Fortunately there are some studies which have shed light on this confusion and have provided clear evidence for both open (contaminated) system behaviour and the existence of anomalous mantle. The recent work of Cortini and van Calsteren¹ is refreshing in that they have studied the genesis of lavas from southern Italy by determining the Pb isotope composition in separated phenocrysts (thought to be crystals precipitated from the magma) as well as in whole rocks. The observation of a systematic isotope disequilibrium led to the conclusion that some open system process has operated, which clearly could not have been demonstrated so unambiguously if the authors had simply analysed the whole rock samples. Remarkably few such

detailed dissections of lavas have been performed previously, although studies of nodule suites^{2,3} have provided incontrovertible evidence for anomalous sub-continental mantle and others have proved the presence of crustal components in plutonic rocks^{4,5}. Having said this, studying minerals separated from volcanic rocks for signs of open systems behaviour is not new⁶⁻⁸, and the evidence acquired for significant isotope disequilibrium has been ascribed to a number of possible causes.

These kinds of studies highlight two important features that could provide the key to understanding the genesis of young volcanic suites. First, detailed studies that include mineral separation and analysis of individual mineral grains and glass, although tedious, are of immense potential value in providing clear evidence of open system processes. One might further envisage that with the development of ion-probe techniques detailed spatial resolution of trace-element concentration and oxygen-

isotope composition will also be an exceedingly powerful way to proceed. Second, much more information on petrogenetic processes can be obtained by using various isotope techniques to study the samples. The elements in silicate systems vary in their mass-transfer response to different conditions and processes. Hence, it is now simply inadequate to study contamination with a single isotope method. It is also a less efficient use of resources to use different samples when using different techniques. Combined isotope studies optimize information with which to define uniquely a signature for a component or process. As there are many igneous provinces for which the crustal contamination versus enriched mantle debate is still unsettled, it is to be hoped that fresh approaches will provide further rigorous testing for the two hypotheses. □

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Tumour immunology

Molecular basis of tumour spread

from Ian R. Hart

METASTASIS, the spread of tumours throughout the body, is a process of obvious clinical significance. Despite the fundamental importance of the phenomenon, relatively little is known about the molecular and cellular mechanisms of its regulation and control. One way in which disseminating malignant cells may evade host defences is suggested in a report on page 301 of this issue, where transfection of neoplastic murine cells with *H-2* genes is accompanied by nullification of their metastatic capacity¹.

Major histocompatibility complex (MHC) class I antigens (termed *H-2K*, *D* and *L* in mice) are distributed widely on nearly all cell types and play an obligatory role in immunoregulation; cytotoxic T lymphocytes only recognize 'foreign' antigens when they are associated with these class I molecules. Immune regulation of tumour growth and development, then, is not solely a consequence of tumour-cell antigens but also depends on MHC class I antigen expression, as demonstrated experimentally in recent studies using DNA transfection techniques^{2,3}. If solid tumour growth can be affected so profoundly by class I antigen expression, how much more likely are metastasizing cells, travelling through the body singly or in small clumps,

to be affected by a similarly regulated immune response? The answer, provided by Hammerling and colleagues¹, seems to be that such effects are just as dramatic.

Two clones from the murine T10 sarcoma, one metastatic and one non-metastatic but both lacking expression of *H-2* molecules encoded by the K-end of the *H-2* complex, were transfected with *H-2K^b* or *H-2K^k* genes. Expression of these *H-2K* antigens in the metastatic clone was found to be associated with a profound decrease in metastatic capacity as determined in immunocompetent recipient mice. Metastatic capacity in immunologically depleted animals remained unimpaired, thus demonstrating the central involvement of the immune response in this phenomenon.

Limitation of metastatic spread by an immune response regulated through MHC class I antigens not only offers hope for potential therapeutic application (interferons, for example, induce a marked increase in the surface expression of these molecules), but also suggests that an inverse relationship exists between malignancy and expression of MHC class I molecules⁴.

This phenomenon may account for the marked heterogeneity that exists for the metastatic phenotype in tumour cell populations⁵. Some findings are consistent

with the existence of such an association; melanoma metastases may lack HLA-DR antigen expression⁶, and malignant mammary carcinomas may exhibit a deficiency in HLA class I antigen expression^{7,8}. Certainly, future studies will test the generality of this association, although unfettered enthusiasm for such an all-encompassing theory on how tumour dissemination occurs must even now be tempered by the knowledge that suppression of MHC class I gene expression is not a universal characteristic of malignant tumours, nor are specific immune mechanisms the only host defence systems capable of modulating metastatic spread⁹. Indeed, increases in MHC class I gene expression may also be associated with metastatic capacity. Earlier studies have shown that expression of the D^k molecule may contribute to the metastatic behaviour of tumour cells¹⁰, and a similar association between metastatic activity and expression of a gene homologous to MHC class I antigens has been established in another murine tumour system¹¹.

Recently the metastatic phenotype has been expressed by Ha-ras oncogene-transformed NIH 3T3 mouse fibroblast cells following transfection with genomic DNA isolated from a human metastatic tumour¹². Acquisition of metastatic capacity correlates with the presence of discrete human DNA fragments, possibly representing a novel gene. Metastatic spread of the Ha-ras-transformed cells occurred in immunoincompetent (irradiated) nude mice but not in immunocompetent, histocompatible mice; the metastatic transfectants are capable of metastasizing both in immune

depleted and in immunocompetent mice. Presumably the transfected gene confers some protection against the immune system and an attractive possibility is that this gene could turn out to be homologous to the putatively suppressogenic *H-2D¹*¹¹.

Unfortunately, such attempts to seek a single unifying explanation for the regulation of metastatic spread are probably oversimplifications; the complex multifactorial nature of the phenomenon suggests that answers obtained are likely to be as varied as the models used to address the questions. The true significance of Hammerling *et al.*'s report¹, along with other recent studies, may lie in the fact that these investigations represent the first applications of the techniques of modern molecular biology, such as gene transfer, to the central problem of clinical cancer and to a still major conundrum of tumour biology. □

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IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

It is still unknown whether the recent findings are relevant to scrapie, illustrated here by a Cheviot sheep rubbing compulsively against a fence (photo courtesy of AFRC and MRC Neuropathogenesis Unit, Edinburgh, UK).

cloning and sequencing of the mRNA for PrP 27-30 and show it to be the product of a single cellular gene. In addition, specific mRNA for PrP 27-30 is found in normal as well as in infected tissue. Can molecular biologists now heave a collective sigh of relief that the central dogma is still intact and that proteins do not self-replicate? For the time being the answer is probably yes, because unless the protein in infected tissue is subtly different to that in normal tissue (as has been found for certain cellular oncogenes), then it seems unlikely that PrP 27-30 is anything other than a normal cellular glycoprotein, the synthesis or degradation of which may be altered as a result of scrapie infection.

Nevertheless, the clarification of the origin of PrP 27-30 still does not explain the nature of the scrapie agent. In a negative way it does lend tacit support to the more conventional argument for a small scrapie-specific nucleic acid, suggested by the existence of different strains of the agent⁷. Whether this nucleic acid is contained in a small virus particle with a low particle-to-infectivity ratio, or is viroid-like and not encapsidated is not clear. But what is clear, now that PrP has been shown to be the product of a cellular gene, is that the hunt for the real scrapie agent is back with a vengeance. □

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Scrapie

Persistently puzzling prions

from Tim Harris

MANY features of scrapie — a degenerative neurological disease of sheep and goats — including pathological similarities to the human degenerative diseases kuru and Creutzfeld-Jacob disease, suggest that it is caused by a 'slow' virus. The inability to find any typical virus particle consisting of nucleic acid and protein, however, coupled with the unusual resistance of infectivity to agents that normally inactivate nucleic acids, has led to some radical thinking about the nature of the scrapie agent. It has been proposed, principally by Stanley Prusiner and his colleagues^{1,2}, that a new type of proteinaceous infectious particle or 'prion' is involved in scrapie infection (for review, see ref.3). Two recent studies^{4,5}, one reported on page 331 of this issue⁴, have now begun to produce molecular evidence that call this claim into question.

The evidence for the existence of prions stems largely from the fact that scrapie infectivity is proteinase sensitive, and that rod-shaped particles resembling amyloid fibrils are observed to co-migrate with

infectivity in sucrose density gradients. SDS polyacrylamide gel electrophoresis of the proteins in this fraction (where infectivity is considerably enriched with respect to protein) has revealed a single polypeptide, PrP (prion protein) 27-30, of relative molecular mass 30,000; no nucleic acid has been found to be associated with it. Several models for the mechanism of prion replication have been put forward⁶, the most heretical of which are that the protein is back-translated into nucleic acid (as yet achieved only by computer), or that PrP is a template for its own synthesis.

Recently, PrP has been purified and its partial amino-acid sequence obtained. The availability of this sequence for the design of oligonucleotide probes means that, if there is a messenger RNA for PrP, it would be only a matter of time before the isolation of complementary DNA clones. Given the degree of interest in the elusive scrapie agent, it is not surprising that this has happened sooner rather than later.

These molecular studies^{4,5} present the

Heavy-ion physics

Giant nucleus at Darmstadt?

from Joshua Silver

WHEN an energetic very heavy ion passes close to the nucleus of a heavy atom in a solid target, positrons may be spontaneously produced within the electron shells of the atom. Recently, researchers at the heavy-ion facility at GSI Darmstadt, West Germany, have been examining this phenomenon. One particularly exciting possibility emerging from these experiments is that extremely heavy nuclei, with atomic number and mass of about 180 and 470 respectively, might be produced in heavy-ion scatterings. Although such products would be short lived, they might open up the study of atoms with unprecedented properties.

Consider an atom of variable nuclear charge Z . As Z increases, the binding energy of the innermost electrons, $E(1s)$, becomes more negative. Forgetting for a moment the fact that there are no atoms with $Z > 109$, we may calculate $E(1s)$ using the Dirac equation with a Coulomb potential for arbitrary Z . It is found¹⁻³ that for a non-

zero nuclear charge radius, $E(1s)$ falls below $-2m_e c^2$ for $Z \geq 173$, where m_e is the rest mass of the electron and c is the velocity of light. When $E(1s)$ is less than $-2m_e c^2$, the discrete bound electron state becomes degenerate in energy with a three-particle continuum state, consisting of two bound electrons plus an outgoing positron wave.

It was first suggested by Gershtein and Zel'dovich¹ that this 'autoionizing' mode of spontaneous positron production might be observable in heavy-ion collisions. The idea is that for a sufficiently close collision of two very heavy ions, it is possible for the 'innermost' $1s$ electrons of the projectile or target atom to experience a Coulomb field similar to that from a nucleus with $Z \geq 173$, so long as $Z_1 + Z_2 \geq 173$, where Z_1 is the atomic number of the projectile ion and Z_2 the atomic number of the target atom⁴. This approach, however, suffers from a weakness — in the words of Müller⁵, the method is "both a curse and a gift"; the rapid time variation of the Coulomb field during the collision leads to ionization of $1s$ electrons from the collision partners, thereby creating vacancies. This is the "gift", in that spontaneous production of positrons is suppressed by the Pauli exclusion principle if the $1s$ levels are full. However, the time variation of the Coulomb field is also responsible for induced positron production which can mask the spontaneous process. Calculation of the relative probabilities of the spontaneous and induced processes is difficult; for completely elastic scattering along Rutherford trajectories the two processes cannot be distinguished in principle. There is also a contribution to the total positron production from nuclear mechanisms.

Three experimental groups have used the heavy-ion accelerator at GSI Darmstadt for several years to search for spontaneously produced positrons. In the past two years, these groups have observed a new phenomenon that is difficult to explain⁶⁻⁸. For coincident observation of positrons with particles scattered in well-defined angular ranges, and at well-defined ion-beam energies in a narrow interval around the Coulomb barrier, the energy spectra of the positrons produced during a collision can show a distinct 'line' at around 300 keV. Typical spectra are shown in Fig. 1. The width of this line may be used to infer a duration for the collision, simply from Heisenberg's energy-time uncertainty relation. The smallest widths observed so far (about 60 keV) translate into a characteristic time for the positron production process of the order of 10^{-19} seconds. Now it is also possible, from the dynamics of the collision, to estimate the time during which the collision partners are sufficiently close together for the transient 'united atom' Coulomb

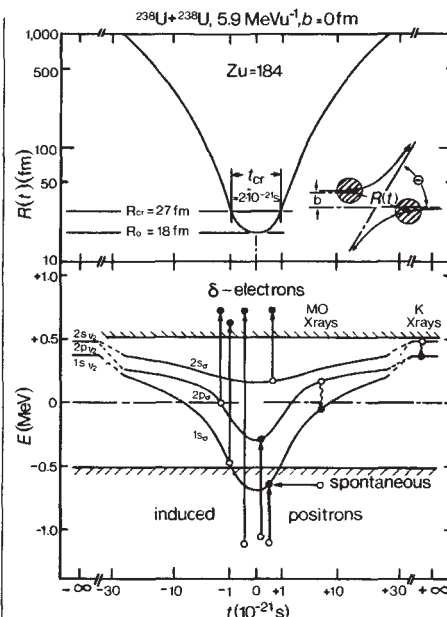


Fig. 2. Electron-binding energies, t , for the three most bound states (top) and the internuclear separation, $R(t)$, for two ^{238}U nuclei as a function of time (bottom) for a collision between a stationary target nucleus and a projectile with energy 1.40 GeV, with impact parameter $b = 0$. Note that $E(1s)$ is $< -2m_e c^2$ for a time of the order of 2×10^{-21} s for this collision (from ref. 10).

field to be large enough to give rise to spontaneous positron emission. For the typical system $^{238}\text{U} + ^{238}\text{U}$ at a beam energy of 1.40 GeV and a head-on collision, this time is $\sim 10^{-21}$ seconds, two orders less than the duration inferred from the width of the positron line (Fig. 2). What is going on?

One possible explanation is the formation, during the collision, of a transient 'giant di-nuclear system' or 'giant nuclear molecule' which lasts for at least 10^{-19} seconds. At present, it is not clear that this explanation is correct or complete, and one problem that is not easily explained is that the most well-defined positron lines are all at an energy of about 300 keV. Nonetheless, the possible creation of an (albeit transient) nuclear system with an atomic mass of about 470 and an atomic number of around 180 is most exciting and has triggered more experimental and theoretical work, in an attempt to understand what is really happening. With the increasing availability of heavy-ion beams of suitable energy it is likely that this phenomenon will soon be better understood.

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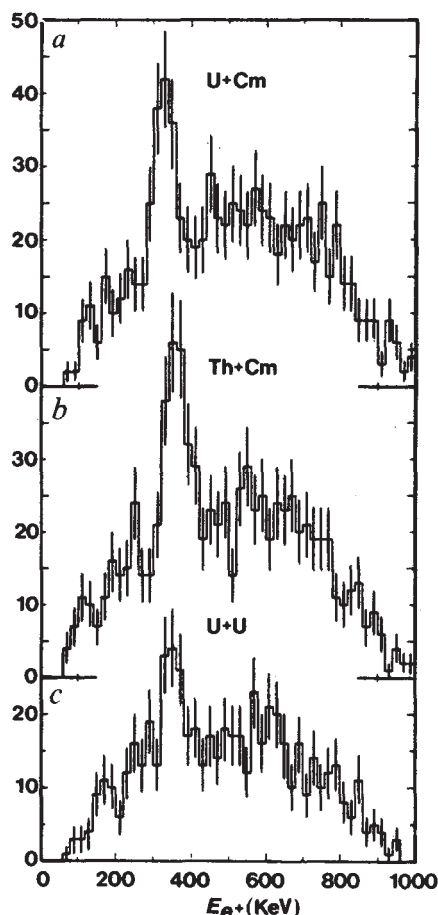


Fig. 1 Energy spectra of positrons produced from collisions of a, ^{238}U ($Z = 92$) on ^{238}Cm ($Z = 96$); b, ^{232}Th ($Z = 90$) on ^{238}Cm ($Z = 96$); c, ^{238}U ($Z = 92$). Bombarding energies are $\sim 6 \text{ MeV A}^{-1}$, and the angular range (of the order of 100) of the ions scattered in coincidence with the detected positrons gives the most pronounced $\sim 300 \text{ keV}$ positron 'line' in each case (from ref. 7).

Visual cortex

Stars and stripes present the colours

from Ian Thompson

THE primate prestriate cortex contains several visual areas, each with its own representation of the visual field and a particular set of receptive-field properties. Their major input arises from striate cortex (V1, sometimes termed area 17) either directly, or indirectly via the projection from V1 to V2. How, then, is the observed diversity of function generated by the input from V1 and V2? Does it depend on specific intra-cortical processing within each area or does it arise because each area receives a functionally specific input? Two papers on pages 322¹ and 325² of this issue and one in the press³ provide complementary information on how anatomical and functional inhomogeneities within one visual area (V2) are related to the neuronal projection pattern between V2 and areas in the prestriate cortex concerned with colour (V4) and movement (termed V5 or MT).

Neurons in V4 and in V5 (MT) display marked differences in some of their stimulus specificities. In V5 (MT), they are selective for the speed and direction of a moving object, irrespective of its colour⁴⁻⁶. The responses of cells in V4 are much more dependent on the spectral properties of the stimulus^{5,6} and may display some form of colour constancy⁷. How do these differences arise?

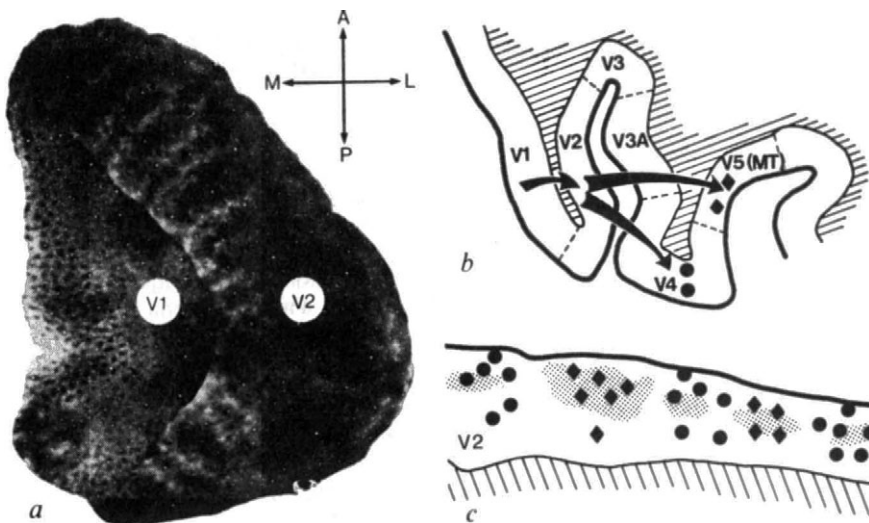
One possibility is that there are separate populations of neurones in V2 projecting to the two areas. Using retrogradely transported tracers injected into V4 or V5 (MT), Shipp and Zeki¹ have demonstrated bands of labelled neurones in V2 that lie orthogonally to the V1/V2 border. Stripes with this orientation in V2 are also seen with another neuroanatomical method, staining for the distribution of the enzyme cytochrome oxidase (part *a* of figure), but Shipp and Zeki note that the spacing of the bands of labelled neurones is twice that of the cytochrome oxidase stripes. In favourable sections, especially from infragranular layers of macaque cortex or from squirrel monkeys, the dark cytochrome oxidase-positive stripes appear alternately thick and thin, separated by light (cytochrome oxidase-negative) interstripe zones. Where identification of thick and thin stripes was possible, Shipp and Zeki report that neurones labelled after a V5 injection coincide with thick cytochrome oxidase stripes, whereas cells labelled from V4 are associated with thin stripes or, occasionally, with the interstripe region (figure *b,c*). They conclude that the output neurones from V2 to V4 or to V5 (MT) are clustered into alternating bands. This conclusion is elegantly confirmed by DeYoe and Van Essen³ who use two tracers that fluoresce at different wavelengths, one injected into V4 and the other into MT (V5).

In V2, adjacent bands are labelled with different tracers and no cells are obviously labelled with both.

Given the anatomical heterogeneity in V2, is there any corresponding grouping in the physiological properties of its neurones, as suggested by an earlier 2-deoxyglucose study⁸? Using electrophysiological techniques, all three groups¹⁻³ describe cluster-

smaller than that of its receptive field. An optimally sized spot is thus equally effective in several positions in the receptive field, a characteristic of complex cells. Hubel and Livingstone postulate that this type of receptive field represents a comparable transformation with that occurring between oriented simple and complex cells. The 'non-complex' double-opponent cells are first seen in V1, possibly reflecting a convergence of afferents from the lateral geniculate nucleus in the thalamus, analogous to that generating oriented simple cells⁹.

The existence of distinct anatomical pathways within V2 projecting to V4 or to



Schematic summary of connectivity patterns within pre-striate cortex taken from refs 1-3. Photomicrograph *a* shows the distribution of cytochrome oxidase staining in a tangential section through V1 (area 17) and V2 (area 18) of squirrel-monkey cortex. A characteristic neuronal connectivity pattern exists between the spots in V1 and the stripes in V2. *b*, V2 projects both to V4 and to V5 (MT) with the cells of origin of both projections clearly segregated into alternating bands in V2 (*c*), depicted by the circle and diamond symbols. An association between the cells of origin and the thick and thin cytochrome oxidase stripes (stippling) is also described in *c*.

ing of receptive field characteristics such as colour, orientation and directionality. Unfortunately, the reports differ in the details of the relation between the clusters and the stripes. One contributory problem seems to be reliable identification of thick and thin stripes. DeYoe and Van Essen³ minimize the problem by combining quantitative electrophysiology, fluorescent tracers and cytochrome oxidase staining. They describe an association of colour selective units with the distribution of cells projecting to V4, which they find can arise from both the thin stripes and interstripes.

The suggested association of colour cells with thin cytochrome oxidase stripes in V2 is interesting because these stripes are thought to be the termination site of the projection from the cytochrome oxidase blobs in V1⁹. Having found previously that V1 blobs were associated with non-oriented double-opponent colour cells⁹, Hubel and Livingstone² now describe a receptive-field type, associated with thin stripes in V2, which they suggest represents an hierarchical elaboration of the V1 double-opponent cell. The new type is also non-oriented and double-opponent but shows spatial summation over a region

MT (and possibly also to other areas³) raises several interesting questions. How is the visual image represented in each pathway? Does each contain a separate map or is the scatter in the topography of V2 sufficient to ensure adequate representation in each projection? Magnification-factor data would be welcome. The anatomical sub-regions are large with the repeat distance from thin stripe to thin stripe between 2.5 and 5 mm. Thus, finer-scale functional representations are possible in the sub-regions, indications of which can be seen in the 2-deoxyglucose results⁸. In fact, it would be surprising if all neurones within a sub-region were functionally identical. Quantitative investigation of distributions of response properties should generate a clearer picture of visual analyses performed in the different visual regions.

Finally, what developmental mechanisms generate the anatomical and functional diversity? The pattern of retrogradely labelled cells in V2^{1,3} is reminiscent of the banding of afferent terminals seen when two projections map onto the same neural sheet, for example, ocular dominance columns in striate cortex or in the tectum of

three-eyed frogs¹⁰. Are the two patterns analogous, providing a solution to the problem of accommodating two maps in one structure? If separate maps do occur in one

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area, then there is a problem with the idea of a one-to-one relationship of function to cortical area. Pursuing the ocular-dominance column analogy, do the projection bands develop from an initially uniform distribution? Recent results from the cat are suggestive — Price and Blakemore have found that cells projecting from area 17 to area 18 initially have a continuous distribution that becomes patchy during development¹¹. □

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Protein crystallography

A reaction centre elucidated

from Jim Barber

PHOTOSYNTHETIC organisms contain specialized proteins — reaction centres — that function to convert the energy of an absorbed photon into the chemical potential necessary to synthesize organic molecules. In all classes of photosynthetic organisms the reaction centre is embedded in a membrane matrix with closely associated pigmented proteins acting as light collection systems. Photons absorbed by these light-collecting proteins cause excitations in the pigments, which are efficiently transferred to the reaction centre. On arrival at the centre the excitation causes a rapid charge separation, the primary step in the conversion of light energy to chemical potential.

This charge-separation process has some remarkable features which have not yet been reproduced in artificial photochemical systems. The most striking features are the speed of the process, which occurs within a few picoseconds, and its very high efficiency. Although some elegant experimental and theoretical studies have attempted to elucidate the mechanisms by which reaction centres bring about energy conversion, progress had been hampered by the unknown molecular organization of components of the protein. Such restrictions have now been lifted because of the success of a group working in Munich, who have crystallized the isolated reaction centre of *Rhodospseudomonas viridis*.

R. viridis is a gram-negative non-sulphur photosynthetic bacterium first identified only twenty years ago. It contains bacteriochlorophyll-*b* (BChl-*b*) as well as several different carotenoids. The bulk of these pigments make up the light-collecting system but a small percentage of the BChl-*b* and carotenoids is found in the reaction centre protein. The precise composition of the *R. viridis* reaction centre was not known until it was isolated as a complete functional entity by J. Thornber *et al.* (*Biochim. biophys. Acta* **172**, 351; 1969). It was soon established that this reaction centre contained four BChl-*b* molecules, two of which form a 'special pair'. These are able to undergo photo-

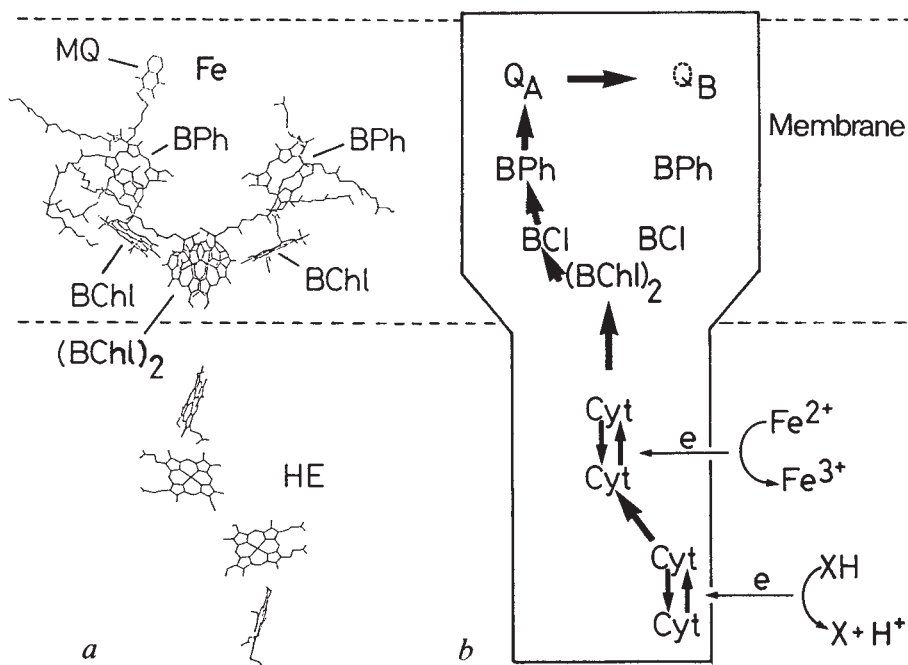
oxidation, detected as an absorption decrease at 960 nm (P960). The isolated protein also contains two molecules of bacteriopheophytin-*b* (BPhe-*b*), two quinone molecules (menaquinone and ubiquinone), a non-haem iron atom and four cytochrome *c* molecules, two with absorption peaks at 553 nm (C553) and two at 558 nm (C558). Gel electrophoresis indicated that the isolated protein is a complex consisting of four different polypeptides of relative molecular mass (M_r) 38,000, 33,000, 28,000 and 24,000. The last three

polypeptides are analogous to those in the reaction centres of other purple bacteria and have been designated H (heavy), M (medium) and L (light) subunits, respectively. The M_r 38,000 component is only found in the *R. viridis* reaction centre and is the polypeptide that binds C553 and C558.

Since the study of Thornber *et al.*, the composition and spectroscopic properties of the *R. viridis* reaction centre have been established using a range of techniques including electron spin resonance, nuclear magnetic resonance, picosecond absorption spectroscopy, fluorescence transients and linear and circular dichroism. As a consequence of these investigations new theories of electron tunnelling and charge stabilization have emerged and structural models have been postulated.

Three years ago, H. Michel successfully obtained crystals of the *R. viridis* reaction centre suitable for high resolution X-ray diffraction analysis which maintained photochemical activity (*J. molec. Biol.* **158**, 567; 1982). In collaboration with J. Deisenhofer, D. Epp, K. Miki and R. Huber, he has now been able to determine the structure of these crystals to a 3 Å level of resolution (*J. molec. Biol.* **180**, 385; 1984). The structural details confirm predictions from spectroscopic measurements as well as revealing some unusual features, still of unknown meaning.

According to X-ray analysis, the protein



Models for the reaction centre of *Rhodospseudomonas viridis*. *a*, Relative positioning and orientation of the prosthetic groups of the reaction centre showing the special pair (BChl)₂, two monomeric bacteriochlorophyll-*b* (BChl), two monomeric bacteriopheophytin-*b* (BPh), one non-haem iron (Fe), one menaquinone (MQ) and the four cytochrome haems (HE). The central local symmetry axis runs vertically in the plane of the picture. Broken lines suggest the position of the membrane whose plane is assumed to be orientated perpendicular to the central local symmetry axis. (Modified from Deisenhofer *et al. J. molec. Biol.* **180**, 385; 1984.) *b*, Schematic drawing of the reaction-centre complex showing electron-transport pathways. Q_A, menaquinone; Q_B, ubiquinone, which is lost during crystallization. The special pair (BChl)₂, BChl, BPh and Q_A are located in a cylindrical part of the reaction centre. Bound cytochromes (C553 and C558) occur as high potential haems, assumed to be reduced to soluble cytochrome c₂, (Fe²⁺ → Fe³⁺) and in closest proximity to the special pair, as low potential haems, which can presumably be reduced by organic acids (XH).

complex has an elongated shape with three clearly distinguishable regions. The central portion consists of the L and M polypeptides and is a 50-Å long cylinder with an elliptical cross-section of diameters between 50 and 70 Å. To each flat face of the cylinder, a globular subunit is attached so that the longest dimension of the entire reaction-centre complex is about 140 Å. One of the globular subunits contains the four haem groups of cytochromes C553 and C558, and the central cylinder is the site for the four BChl-*b*, two BPh-*b* and a high electron-density peak from a non-haem iron atom. The other globular structure is presumably the H-subunit, known not to be directly involved in the photoactivity of the reaction centre.

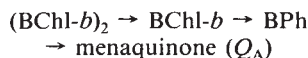
Although the Munich group has not fully elucidated the complete polypeptide structure, it has obtained details of the spatial relationships between various components involved in charge stabilization (see figure). A prominent feature is that the BChl-*b* and BPh-*b* molecules are arranged with an approximate 2-fold rotational symmetry. The rotation axis runs through (or nearly through) the non-haem iron atom and through two closely associated BChl-*b* molecules, whose pyrrole rings are stacked on each other. These two BChl-*b* molecules are the 'special pair' corresponding to P960; the X-ray diffraction data indicate that a linkage exists between the magnesium atom of one tetrapyrrole head and the acetyl group of ring 1 of the other, a conclusion which invalidates an earlier suggestion that water was involved in bridging. The average distance between the atoms of the tetrapyrrole heads is 3 Å with a Mg-Mg distance of 7 Å, and the angle between the ring planes is about 15°. Interestingly, the other two BChl-*b* molecules are symmetrically spaced from the special pair at a Mg-Mg distance of about 13 Å and an angle of 70° between the planes of the tetrapyrrole head groups. Both the monomeric BChl-*b* molecules contact BPh-*b*, with a spacing between ring centres of approximately 11 Å and a 64° angle between ring planes. Although all the tetrapyrrole rings are on the same 2-fold rotational axis, their phytol side chains are not.

The non-haem iron atom is positioned on the 2-fold rotation axis and is linked to at least three histidine residues, one of which has an electron density close to it that is indicative of menaquinone (Q_A). The iron-quinone distance is approximately 7 Å and, significantly, in the symmetry-equivalent position to the quinone ring related by the 2-fold axis, is an empty 'pocket' in the protein. Michel and collaborators suggest that this empty pocket is the ubiquinone binding site termed Q_B . Although the haem groups in the cytochrome subunits do have a symmetrical arrangement, they are not positioned on the 2-fold rotational axis relating the BChl-*b* and BPh-*b* molecules. Only the haem closest to the special pair lies on this symmetry axis with an Fe-Mg distance of about

21 Å. Thus, it may be this cytochrome which directly reduces the photo-oxidized special pair (P960⁺).

From the diffraction analysis it is clear that the L and M subunits form the central cylinder, which consists of five trans-membrane helices, each being related by the 2-fold rotational axis. Because of this, both subunits are equally involved in forming the special pair and the non-haem iron-quinone complex. Both the cytochrome and H-subunits also contain helices.

In many ways the structure determined by X-ray crystallography has supported several concepts derived from spectroscopy. Clearly the organization of the components confirms that primary charge separation involves the following electron transfer sequence:



with $(\text{BChl-}b)_2^+$ being reduced by cytochrome *c* while menaquinone passes its electron to a ubiquinone (Q_B) molecule normally bound in the vacant pocket. The structural model also supports the chromophore orientation data obtained from linear and circular dichroism. The puzzling, as yet unaccountable, feature is the symmetrical arrangement of the two monomeric forms of BChl-*b* and BPh-*b*, which suggests two possible electron transfer pathways. However, evidence suggests that only one pathway is preferred so that an electron is always passed to Q_A , the bound menaquinone, before going to ubiquinone (Q_B). It seems, therefore, that one BChl-*b* and one BPh-*b* molecule are not normally involved in the process of charge separation.

It could be argued that the reaction cen-

tre of *R. viridis* has evolved from a more primitive reaction centre consisting of one subunit only, and that the dimerization giving rise to the L and M polypeptides facilitated the formation of the special pair and the opportunity for improving charge stabilization by allowing the Q_A to Q_B electron transfer to occur. The precise factors which have given rise to the preferred pathways will become clear as the detailed structure of the polypeptide chains are determined from the electron-density map. Moreover, the role of the H-subunit should become more obvious as Deisenhofer and colleagues continue to elucidate the total structure of the protein complex.

In any event, it is probable that the molecular details of the *R. viridis* reaction centre will not only allow the theoreticians to understand how the high efficiency of energy conversion is attained in this specific protein, but will also act as a model for the reaction centres of other organisms. In fact the *R. viridis* reaction centre is structurally and functionally similar to the reaction centres of several purple non-sulphur bacteria. Even more exciting is that the knowledge gained from these studies will form an important basis for elucidating the molecular basis of the mechanism of water splitting by green plants and algae, because these reactions occur at a reaction centre (photosystem two) having features similar to those of the purple bacteria. We can also look forward to a future where X-ray crystallography will unravel the details of a wide range of membrane processes at the resolution of atomic distances. □

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Palaeoclimatology

Records of past continental climates in deep-sea sediments

from Joseph M. Prospero

EVERYONE talks about palaeoclimate, but no one seems to know much about it. This is especially true for continental climates where the often subtle evidence of past weather conditions is easily obliterated by subsequent events. Even when evidence is found, in many cases it is not possible to date the events. Ocean scientists are more fortunate because a good chronological record of past oceanic conditions (such as water temperature) can often be read in the neatly ordered layers of deep-sea sediments that span many millions of years. Recently, Rea *et al.* (*Science* 227, 721; 1985) have shown that these sediments also contain information about past continental climates. Their conclusions about atmospheric circulation over the past 70 million years not only challenge conventional views on palaeoclimates, but also provide a test for

computer climate models.

Rea *et al.* analysed the windborne (aeolian) mineral dust components in four North Pacific sediment core sections spanning the past 70 Myr and found major changes in particle size, concentration and the mass accumulation rate. They suggest that the accumulation rate data are indicative of the availability of material in the source area and should be related in some way to climate, especially to aridity. The mineralogy of the aeolian grains also provides clues to the climate of the source region and, most importantly, the size distribution can be used to infer the speed of the transporting winds.

On the basis of their assumptions, the authors conclude that the atmospheric circulation was quite vigorous during the late Cretaceous and Palaeocene, but moderated

sharply in the early Eocene (50 Myr ago) before commencing a steady increase that culminated in a maximum associated with the Pliocene-Pleistocene glaciations of 4–2 Myr ago. The most surprising outcome of this work is the implication that circulation during the late Cretaceous was as vigorous as that in the Palaeocene. This is contrary to the long-held belief of geologists and palaeoclimatologists that the Cretaceous was a period of moderate climate and relatively weak circulation, but is supported by recent computer models.

Data such as that of Rea *et al.* are important as relatively rigorous tests of long-term climate models, which otherwise would be little more than interesting intellectual exercises. It is therefore necessary to scrutinize the underlying assumptions of such studies. The most critical assumption is that which relates aeolian particle sizes to the speed of the transporting wind. Rea *et al.* assume that, for any one wind speed, there is a characteristic particle-size spectrum of dust grains which are in 'equilibrium' with the wind, where the 'lifting effect of the inherent turbulence must balance the tendency of the grains to settle'. On this basis, the ratio of the transporting wind speeds for two different events will be equal to the ratio of the squares of the 'equilibrium' particle diameters.

The concept of a quasi-stationary size distribution related to wind speed is based on work by Gillette *et al.* (*J. geophys. Res.* **85**, 5621; 1980) who studied soil deflation as a function of soil properties and boundary layer micrometeorology. It is, however, debatable whether boundary layer turbulence processes can be extrapolated to the free troposphere where vertical transport and mixing is largely dependent on processes occurring in large-scale weather systems and during convection. Although Rea *et al.* cite some experimental evidence that they believe tends to support their assumption, there are no studies that directly address the relationship of particle size to the speed of the transporting winds.

Even the relationship between soil deflation and aridity is complex. Goudie (*Prog. phys. Geog.* **7**, 502; 1983) has shown that the correlation between dust storms and rainfall is very weak; indeed, the frequency of dust storms is at a maximum in regions with 100–200 mm annual rainfall. This is consistent with growing evidence that water is essential for the production of abundant quantities of fine sedimentary particles (Pye, K. and Sperling, C.H.B., *Sedimentology* **30**, 49; 1983) and for creating surficial environments where these weathering products can be deflated readily (Gillette *et al.*, *op. cit.*). In contrast, many hyperarid areas are relatively blown out; in such regions, the principal features of the surface are rocky plains and dune fields which are primarily comprised of coarse materials that are not readily deflated or transported great distances by winds. These factors suggest that the most abundant

sources of soil dust are areas that are changing from moist to arid. This is supported by Prospero and Nees (*Science* **196**, 1196; 1977 and *EOS* **65**, 837; 1984) who demonstrated a threefold increase in the transport of dust out of North Africa and across the Atlantic during both the current drought and that of the early 1970s.

Another aspect that must be considered is the relationship between the meteorology of dust storms and weather. Studies of dust storms in Africa, North America and Asia suggest that if climate were to change, weather would also change and, with it, the sources of the dust, the mechanism by which it is generated and its transport paths. Sediments in the North Pacific would be particularly sensitive to changes in transport paths. Recent atmospheric chemistry studies in this region (Uematsu *et al.* *J. geophys. Res.* **88**, 5343; 1983) show that Asia is the primary source of the large quantities of dust found in North Pacific winds; it is carried out of Asia on westerly winds and, eventually, some fraction is transported via complex trajectories into the easterlies. As a consequence, dust concentrations decrease markedly in the latitudes south of about 30° N. Therefore, it is conceivable that dust deposition rates in some ocean regions could have changed

substantially solely as a consequence of changes in circulation patterns.

Finally, the fact that large quantities of dust could have been produced by climatic factors not directly related to aridity must be considered. Most significant is the considerable evidence (Pye, K. *Prog. phys. Geog.* **8**, 176; 1984) that extensive areas of loessal soils found in North America, Europe and Asia have been formed through the combined action of winds and glaciation. The dearth of the fine particles in these deposits suggests that large quantities of fine soil dust were blown away, although the source region was not necessarily arid.

It is clear that deep-sea sediments contain a strong and varied aeolian signal that must be linked in some way with climatic processes on the continents. Rea *et al.* have taken the first step towards interpreting this signal. Whether their interpretation is correct will be established only by further studies of the areal and temporal variation of this signal in the sediments together with studies of the present-day relationship between weather and processes of dust mobilization and transport. □

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Parasitology

Chromosomes of malaria parasites and trypanosomes

from F.E.G. Cox

OUR understanding of the genetics of the protozoan parasites that cause major diseases in man has been hampered by the fact that we do not even know how many chromosomes they possess. On page 347 of this issue, Kemp and coworkers report the use of a new technique, pulsed-field gradient (PFG) gel electrophoresis, to separate and characterize seven chromosomes from the human malaria parasite *Plasmodium falciparum*¹ (see figure). This technique has enabled several groups not only to enumerate such elusive chromosomes but also to separate and identify them and to elucidate some of their functions. It has already been used to separate 11 of the 17 chromosomes of the yeast *Saccharomyces*².

PFG gel electrophoresis involves the separation of large molecules of DNA in an agarose gel³, and the DNA obtained from the lysed organisms is subjected to alternate electrical pulses at right angles. Because these molecules are larger than the pore size of the gel, they have to orientate themselves end on to pass through the gel; their ability to reorientate at right angles and the time taken to do this provides a basis for separating the molecules. The DNA thus separated can be visualized by staining and blotting on nitrocellulose and

the genes located by hybridization techniques. In this way, it is possible to locate and identify DNA molecules in the size range of 30–3,000 kilobases (kb), which happens to include most of the DNA constituting the chromosomes of malaria parasites and trypanosomes.

All that was previously known about the chromosomes of *P. falciparum* was that there are few of them and that they seem to be haploid in the blood stages⁴. Kemp *et al.*¹ studied three strains of *P. falciparum*, one from Papua New Guinea, which was used as a baseline, and others from Ghana and Thailand. Using lysed cultured blood-stage parasites, they found that their DNA separated out into seven discrete zones and, by using yeast chromosomes as markers and complementary hybridization data, they established that these represent complete chromosomes. The four smallest chromosomes were the easiest to identify and, in the Papua New Guinea strain, consist of 0.8, 1.2, 1.6 and 1.8 megabases (Mb). Chromosomes 5–6 were 2.2 and 2.6 Mb and chromosome 7, which overlapped with 6, was 3.0 Mb. The other strains of *P. falciparum* also had seven chromosomes but exhibited considerable polymorphism with respect to the Papua New Guinea strain. For example chromo-

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Plasmodium falciparum in human red cells in culture. *P. falciparum* grows easily in culture, so is readily available for laboratory study. In this stained preparation, the nucleus appears as an amorphous dark mass with no detailed structure. Chromosomes can only be identified after separation by pulsed-field gradient gel electrophoresis. (Photograph A R Dluzewski.)

some 1, the smallest, varied between 0.8 and 1.0 Mb and others differed by as much as 20 per cent. There was also variation between the positions of the chromosomes in the agarose gel, but overall a common sequence of seven equivalent chromosomes of increasing size could be identified in each strain. There may also be other chromosomes, probably clustered towards the larger end of the size range where separation presents particular difficulties.

Having separated the chromosomes, hybridization techniques can be used to assign functions to them. The most striking gene products to be identified so far in malaria parasites are the short repeats of amino acids that constitute important antigens. Overall, it seems that the genes for these repetitive antigens are distributed between several chromosomes.

The antigen located on the surface of infected erythrocytes⁵ is encoded by chromosome 1 and also, to a lesser extent, by chromosome 7; the secreted (S) antigen⁶ is also associated with chromosome 7. It is of particular interest that five other repetitive antigens are associated with both chromosomes 5 and 6. Analysis of the results of hybridizations suggests that chromosome 5 is an exact but incomplete copy of chromosome 6 and that this chromosome, 5-6, is diploid, whereas chromosomes 1-4 are haploid.

The chromosomes of trypanosomes have not revealed their secrets so easily. The DNA of trypanosomes is divided between the nucleus and a unique organelle, the kinetoplast, which considerably complicates the study of the genetics of these organisms. Nevertheless, PFG gel electrophoresis is beginning to produce clues about the genes encoding the variant surface glycoproteins (VSG) that coat the trypanosome and are the basis of its ability to evade the immune response by antigenic variation.

Conventional agarose separation of the DNA of *Trypanosoma brucei* produces two fractions, a minichromosome band and the remainder of the DNA that stays at the starting position. PFG gel electrophoresis separates *T. brucei* DNA into four components, a minichromosomal fraction

containing about 100 DNA molecules in the 50-150 kb range, about 6 chromosomes of 200-700 kb, 3 or more larger chromosomes of 2 Mb and a large heterogeneous fraction that hardly migrates at all from the starting point⁷. The genes encoding the VSG seem to be distributed among several chromosomes and are seen most readily in the minichromosomes. Recombination involves the transposition of large numbers of base pairs from one chromosome to another, resulting in major changes in the size distribution of the minichromosomes. It is possible to relate these size changes to changes in the expression of particular VSG genes, which cannot be done using more conventional techniques⁸. This leads to the question of whether minichromosomes are essential for antigenic variation. To answer this, van der Ploeg and colleagues have made a comparative study of several species of trypanosomatid flagellates⁹. PFG gel electrophoresis reveals tremendous differences between the chromosomes of different species. In contrast to *T. brucei*, *T. equiperdum* has few minichromosomes and *T. vivax* has no DNA smaller than 2,000 kb (ref. 9); as both can undergo antigenic

variation it seems that minichromosomes are not essential for this process.

For a long time, parasitological problems have been emerging faster than the techniques available for their solution, which has resulted in frustrating gaps in our knowledge. PFG gel electrophoresis will quickly resolve some of these questions; the application of this relatively simple technique represents a milestone in modern parasitology. It should soon be possible to distinguish between genes encoding antigens important in protection and those used by parasites to evade the host immune response. It might also be possible to distinguish between virulent and avirulent strains of parasites and to pinpoint the basis of drug resistance. □

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Geomagnetism

On the trail of Earth's tail

from Stanley W.H. Cowley

NEARLY all of the planetary bodies in our Solar System are believed to possess long magnetic tails extending for many tens to hundreds of planetary radii in the direction away from the Sun, but none has been so thoroughly investigated as that of the Earth. That has been particularly so since 1983 when the ISEE-3 spacecraft was sent on a series of passes extending about 230 Earth radii along the geomagnetic tail. Altogether, ISEE-3 spent around 8 months beyond the lunar distance, and provided unique and detailed data on the distant tail and its environs. Twenty papers arising from initial analyses of these data have recently been published in an issue of *Geophysical Research Letters*. These papers deal principally with the overall structure of the tail and the properties of plasmas and fields both in the magnetic tail lobes and in the central current sheet that separates the lobes.

Magnetic tails are formed by an interaction with the solar wind, the tenuous hydrogen plasma which blows continuously outwards from the Sun at speeds of around 500 km s⁻¹. In the case of a magnetized planet, such as Mercury, Earth, Jupiter and Saturn, the tails are thought to result from magnetic reconnection on the dayside of the planet, a process which produces 'open' magnetic field lines mapping directly from the planet's polar regions into the solar wind. The flow of the wind then carries the field lines downstream from the planet and stretches them out into a nightside tail con-

sisting of two bundles of oppositely directed magnetic flux (mapping to either pole) squashed together into a long cylinder. The field lines in the two tail lobes eventually reconnect across the internal interface between them, returning 'closed' field lines to the planet and 'disconnected' field lines to the solar wind.

Non-magnetized planets such as Venus and probably Mars also possess magnetic tails, but in their case the solar wind plasma becomes slowed on the dayside of the planet by its interaction with the planet's ionosphere. Field lines embedded in this slowed plasma become draped around the planet and stretched into an 'induced' tail as their distant 'ends' continue to move with the wind away from the Sun. A similar process is thought to be responsible for the formation of Type 1 comet tails. The phenomenon of magnetotail formation is therefore very common in our Solar System.

Although these basic notions have been commonly accepted for a number of years, detailed spacecraft observations of magnetotail plasmas and fields had been very limited before ISEE-3. Even in the case of the Earth's magnetotail, most observations had been confined to the region within the lunar orbit (60 Earth radii), a distance representing only about one-twentieth of the tail's theoretically estimated length. The papers in *Geophysical Research Letters* show how drastically the situation has been altered by the travels of ISEE-3.

Two of the papers demonstrate in very

different ways that the field lines in the tail lobes are indeed magnetically 'open', that is they eventually map across the outer surface of the tail into the surrounding solar wind. Changes in plasma bulk parameters, such as density, temperature and velocity, during ISEE-3 traversals of this boundary are reported by Gosling *et al.*¹, who find that well-defined transitions in the magnetic field (sharp changes in field magnitude and direction) are often accompanied by gradual variations in plasma parameters. These observations indicate the presence of direct magnetic flux linkage across the boundary, allowing solar wind plasma a relatively easy access. Abrupt plasma changes also occur, indicating a locally magnetically closed surface.

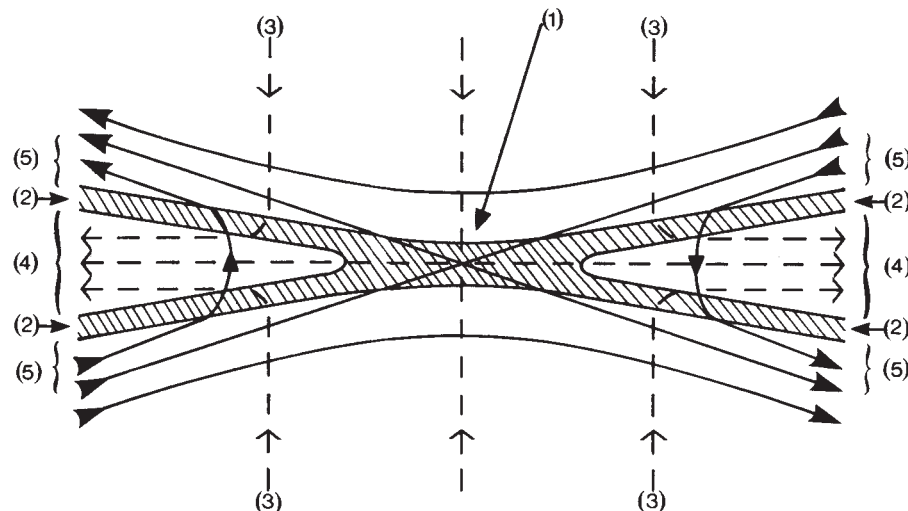
The second paper deals with the direction of the field in the tail and its dependence on the solar wind field direction, measured simultaneously upstream from the Earth by the IMP-8 spacecraft. Tsurutani *et al.*² find that the values of that cross-tail field component that lies approximately parallel to the central current sheet are positively correlated with the corresponding component in the solar wind. In effect, the lobe field (and that in the central current sheet) does not point directly along the tail axis towards or away from the Earth, but is somewhat skewed towards the direction of the solar wind field. This is precisely the result expected for an 'open' tail structure³, arising from the effects of the magnetic stresses exerted on the plasma by the open field lines.

Equally interesting discoveries have been made about the hot plasma sheet particle population that carries the cross-tail current between the two magnetic lobes of the tail. A previous article in these columns⁴ described the initial finding that beyond about 100 Earth radii, the flow of this plasma is directed almost exclusively away from the Earth, indicating that the tail reconnection region usually lies less far from Earth. The appearance of closed field loop 'plasmoids' in the distant tail about 30 min after the onset of magnetically disturbed periods has provided evidence of rapid tail reconnection near the Earth. Confirmation is provided by several papers describing energetic-particle, plasma and magnetic field observations and their relationship⁵⁻⁷.

100 Years Ago

THE verdict of the jury who considered the case of the Usworth Colliery explosion is probably the first expression of opinion from a public body of this class that coal-dust and a small percentage of fire-damp can play the part that has hitherto been usually ascribed to fire-damp alone. They found that the explosion was caused by a shot, the fire of which acted upon "the coal-dust and a small percentage of gas." The convenient and time-worn "outburst of gas" theory, which consigned the helpless miner to the vicissitudes of chance and exonerated colliery owners and their agents from all responsibility, seems on the point of giving way before the coal-dust theory.

From *Nature* 32 61, 21 May 1885



The reconnection geometry of Earth's geomagnetic tail suggested by recent ISEE-3 observations. Broken lines are plasma streamlines, solid lines are magnetic field lines, and hatched areas represent regions of high electrical current density. A single current layer or diffusion region (1) at the site of reconnection bifurcates into slow mode shocks (2) in the downstream flow. Plasma flowing from the tail lobes (3) is accelerated across the shocks to form a hot plasma sheet (4) that flows away from the reconnection site on either side. Suprathermal particles, escaping upstream of the shocks, form boundary foreshock regions of streaming particles (5) located on reconnected tail-lobe field lines just outside the plasma sheet. (No attempt has been made to represent the asymmetries that would result from the observed flow of lobe plasma away from the Earth.)

An important subsequent discovery is that the interface between the tail lobe and the plasma sheet often has the character of a slow-mode shock wave⁸. The formation of such shocks resulting from magnetic reconnection was suggested by Petschek⁹ over 20 years ago, but ISEE-3 has provided the first evidence for them. The field and plasma structure implied by the observations are shown in the figure. The single current layer separating the tail lobes at the site of reconnection (the 'diffusion region', where the field diffuses through the plasma) bifurcates on either side into two slow shocks standing in the plasma flow. Tail-lobe plasma flowing across the shocks is accelerated away from the reconnection region by the magnetic stresses to form a hot accelerated plasma (the plasma sheet) which is mainly confined between the shock waves¹⁰. However, high-speed ions and electrons are observed to escape upstream of the shocks to form 'boundary layer' or 'foreshock' regions of streaming supra-thermal particles, located on tail-lobe field lines between the surface of reconnected field lines mapping to the diffusion region and the shock waves (see figure)^{11,12}.

Scarf *et al.*¹³ describe a well-defined plasma wave morphology associated with these structures. Electron plasma oscillations of a few kHz are observed in the foreshock region in association with a 'heat flux' formed by field-aligned streaming electrons that have energies above a few hundred eV. These waves therefore probably result from the Landau 'bump-on-tail' instability. A similar association seems to occur in this region between streaming energetic ions (of tens of keV) and the magnetosonic waves reported by Tsurutani and Smith¹⁴. Within the shock current sheet itself, a spectral peak in electric field noise at frequencies of a few hundred Hz, possibly identifiable as ion

acoustic waves, is found¹³. An estimate of particle scattering by these waves suggests that they do not lead to appreciable resistivity; instead the lower hybrid drift instability (at too low a frequency to be measured by ISEE-3) may play an important role¹³. In my view, another important factor determining the structure of the current sheet is likely to be finite ion inertia.

An important question raised by these findings is whether the boundary of the plasma sheet on the earthward side of the tail reconnection region also has the nature of a slow shock. It would be surprising if this were not so in the more distant tail, but the near-Earth regime will be complicated by two factors. First, field line convergence at the Earth mirrors accelerated particles back in to the tail. Second, it is possible that the earthward-streaming plasma observed at the outer surface of the near-Earth plasma sheet predominantly results not so much from local acceleration as from the denser tail-lobe plasma found at somewhat larger distances. A deeper understanding of magnetotail physics will undoubtedly ensue from these and other considerations of the consequences of the ISEE-3 observations. □

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The quest for pure electron lattices

SIR — The very interesting work on observing three-dimensional electron lattices, reported in *News and Views*¹, is even more widely relevant than discussed in the brief space available. We note here other laboratory experiments and also, perhaps surprisingly, some astrophysical theory. An essential point of departure stems from the (correct) observation in the article that it is "taken for granted that an electron lattice can form only if embedded in a neutralizing sea of positive electric charge". An electron lattice can be formed *without* such a neutralizing background.

It has been known for years that a magnetized Penning trap can stably store single charged particles for extremely long periods. Such a trap consists simply of an axially symmetric electric quadrupole (two negative electrodes with a positive ring electrode in between, preferably with hyperbolic cross-sections of the appropriate curvature) and a magnetic field along the symmetry axis. The magnetic field prevents a charged particle from escaping across the magnetic field and the quadrupole electric field confines the particle (if negatively charged for the above charging of the electrodes) along the axis. Until fairly recently, it was not understood what happens if one fills such a trap with a large number of particles. Older literature vaguely discussed 'clouds' of trapped particles. It is now known that the charged particles condense effectively to form what appears very much to be a liquid. That is to say that the particles are confined to a spheroidal volume of constant density with a sharp interface to the surrounding vacuum. These sharp discontinuous surfaces were predicted theoretically for both the laboratory and astrophysical cases^{2,3}. The surface thickness separating the plasma from the vacuum is shown to be only of the order of the Debye length, which is small compared with the system scale in the neutron star case even for million-degree plasmas, and in the laboratory case for the relatively cool plasmas that can be produced there (room temperature or less). Moreover, such systems ('non-neutral plasma' is the usual term in laboratory circles) have been created in the laboratory with both electrons⁴ and ions⁵. Indeed, in the case of the ions, the particles have been cooled to a temperature (0.1 degrees kelvin) where the system should not only appear liquid-like (confinement to a variable shape volume of constant density) but actually be a liquid in the sense of large particle-particle correlations. Future experiments aspire to produce a trapped solid of ions (D.J. Wineland, personal communication). The workers at the University of California at San Diego have trapped the electrons in a similar configuration, using ring electrodes at the ends of a long solenoid that parallels the magnetic field axis. It may be feasible to cool such trapped electrons to tem-

peratures where they are liquidized or solidify⁶. In either case, the formation of an ideal 'theoretician's' lattice of totally non-neutral plasma free of interfering background neutralizing charges seems within reach.

In the case of astrophysics, solidified states of matter have been expected for neutron star interiors (for example, refs 7,8). However, it now seems increasingly possible that the exteriors of magnetized neutron stars may, in many cases, be surrounded by such quasi-liquid non-neutral plasmas pulled from their surfaces. The essential ideas were forwarded by a number of people such as Holloway⁹ who pointed out the likelihood of vacuum-plasma interfaces, Rylov¹⁰ who first speculated on how such plasmas might be distributed about pulsars, and Jackson¹¹ who also argued for condensation of such plasmas about neutron stars. The laboratory effort itself goes back at least to Trivelpiece¹², and an entire book has been written on the properties of nonneutral plasmas¹³.

Not only do these remarkable plasma experiments open up the possibility of creating pure liquid and solid plasmas, but they also underscore an interesting convergence in research on solid-state physics¹⁴, with laboratory experiments on non-neutral plasmas and, surprisingly, some astrophysical theory as well.

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Selfish DNA and the origin of introns

SIR — The selfish DNA or transposon theory of the origin of introns¹⁻⁹ is dismissed far too readily by Cornish-Bowden¹⁰. As introns are very often not at domain boundaries^{11,12}, the hypothesis of evolutionary exon shuffling to recombine protein domains is hardly the "most plausible suggestion" for their "function". It does not adequately explain either the origin or the evolutionary maintenance of RNA splicing. Although fusion of two genes by recombination between existing introns probably has been evolutionarily important, it does not explain the origin of the introns "in the first place". *De novo*

intron formation by fusing two genes lacking introns is far more complex than fusion to produce an unsplit gene; it could hardly have occurred in a single step in an organism previously lacking RNA splicing⁷. Split genes and RNA splicing could not have evolved merely because they might millions of years later help a new protein evolve: evolution lacks such foresight².

The transposon hypothesis can explain the origin of introns and also of RNA splicing itself³⁻⁷. The presence of introns in mitochondria and chloroplasts, in particular, is best explained by transposition from the nucleus⁵⁻⁷. The different positions of the introns of the chloroplast *psbA* genes of *Chlamydomonas*¹³ and *Euglena*¹⁴, coupled with their absence from the ancestral cyanobacteria¹³, supports insertional origin for chloroplast introns, rather than one from primordial gene fusions that created the *psbA* gene. The coding of proteins involved in splicing by certain introns in fungal mitochondria⁴, and in a chloroplast gene¹⁵, supports the thesis that such introns originated as defective transposons in which the RNA-splicing enzymes evolved from pre-existing DNA splicing enzymes specific for their termini³⁻⁵.

This also can explain the origin of introns in general^{3,4,7}. Suppose a transposon capable of precise excision inserted into a cellular gene, thus inactivating its transcript⁴. This first 'intron' would be exactly plied out of the RNA if the DNA excisionase of the transposon mutated so as to be able to cut RNA instead of DNA, but retained its specificity for the sequence at either end of the transposon; this would simultaneously prevent its self-transposition and rescue the cell from its harmful insertion mutation.

This mutation would protect the cell from insertion mutations by any transposons generating similar terminal sequences upon insertion, and therefore have allowed the rapid intragenomic spread of kindred non-defective transposons; moreover these would have provided DNA excisionase activity allowing the rapid intragenomic spread of the defective RNA-splicing transposon itself⁷. So many genes would quickly acquire introns that RNA splicing could never be lost^{1,2}. All descendants of this cell would be permanently infected with useless introns. RNA splicing could later acquire real functions for at least some genes, such as differential splicing to make different proteins from the same transcriptional unit¹⁶. This rescue hypothesis⁴ provides an immediate and powerful mode of spreading for introns and RNA splicing. At one and the same time it involves intragenomic kin selection favouring the spread of selfish DNA, and conventional selection at the cellular level for protection from the harmful effects of certain selfish transposons. Alternatively, RNA splicing might have originated in a virus⁷ before being taken over by the cell.

The transposon theory of intron origins best fits the nuclear mRNA and rRNA splicing mechanisms, both of which depend on specific sequences within, as well as at the end of, introns¹⁷. I suggest that they originated in an early eukaryote and were never present in prokaryotes. The small nuclear RNAs involved in nuclear pre-mRNA splicing might initially have been synthesized from transposon promoters lying on the DNA strand opposite the transcribed strand of the cellular gene into which it had inserted. But nuclear tRNA splicing does depend on specific intron sequences and may have originated independently; the presence of similar tRNA introns in the archaeobacterium *Sulfolobus*¹⁸ suggests that it originated before archaeobacteria and eukaryotes separated. Chloroplast tRNA introns seem unrelated to nuclear and archaeobacterial tRNA introns; they resemble mitochondrial and nuclear rRNA introns and have features suggestive of defective transposons^{6,15,19}.

The improbability of losing every single intron by accidental deletion^{2,4}, and the extra complexity that splicing poses for the origin of protein coding⁴, make it improbable that RNA splicing was the general rule in the primordial cell, and favour the view that archaeobacteria and eukaryotes both evolved from a eubacterium lacking split genes⁴. Once introns evolved, their number and total length per genome would increase or decrease in response to generalized selection for larger or smaller total genome size¹ and transcript size, as well as to selection for specific functions¹⁶ for at least some introns.

The relative uniformity of exon length in protein-coding genes is not, as claimed by Lonberg and Gilbert¹², good evidence against an insertional origin. The periodic structure of chromatin may prevent totally random insertions. The correspondence between the three major peaks in exon size distribution and the DNA lengths in nucleosome core particles, linkers and whole nucleosome²⁰, suggests that the junctions between linkers and core particles have been 'hotspots' for intron insertion and therefore that introns in protein genes originated after the origin of eukaryotic chromatin. By contrast, exon length in mitochondria²⁰ and chloroplasts^{13,14,21,22}, which lack histones, is much less uniform and more random than in nuclei.

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Chemical warfare evidence unconvincing

SIR — Rosen *et al.*¹ fail to substantiate their contention that environmental samples from south-east Asia provide evidence of chemical warfare. They base their argument on reports of trichothecene mycotoxins in six samples from alleged chemical attack sites^{2,3}. In arguing that the toxins they reported were not of natural origin, they astonishingly say that the US Army Chemical Systems Laboratory found no trichothecenes in samples from south-east Asia.

This incorrectly implies that the Army's samples were controls. In fact, at least 60 of the Army's samples, like those of Mirocha and Rosen, were from alleged chemical attack sites. As far as we know, the Army laboratory has not found trichothecenes in any sample from such sites. Mirocha and Rosen have known of this since at least October 1983, when one of us (M.M.) wrote to them asking "Why does the Army find no positives out of 60 samples while Mirocha plus Rosen find six out of six positive?" The evidence for trichothecenes at sites of alleged chemical warfare is inconclusive at best: 6 positives out of 6 samples compared with 60 negatives out of 60 samples.

Rosen *et al.* ignore the fact that it is pollen, and not trichothecenes, which is the consistent and confirmed finding in samples of yellow rain, the alleged chemical warfare agent. Since 1979, many samples have been turned in by Hmong refugees from Laos, and then examined by US and other investigators. In November 1982, the head of the US Army laboratory referred to above stated "most of the samples that are of yellow rain are fairly dry and they have a high level of pollen grains in them"⁴.

To our knowledge, all samples of yellow spots and powders from sites of alleged chemical attack that have been examined under the microscope, including those analysed by Mirocha and Rosen, consist largely of pollen. This is also true of honeybee faeces. Moreover, our analysis of the pollen types in samples of the alleged

agent collected by Hmong in April 1981 and in March 1982, and comparison with pollens gathered by local honeybees, strongly supports the identification of yellow rain as the faeces of south-east Asian honeybees⁵.

Rosen *et al.* also ignore descriptions of the alleged agent by Hmong refugees. Summarizing interviews with Hmong for the period 1978-82, the official reports of Secretaries of State Alexander Haig and George Shultz state that the alleged agent is yellow and falls like rain^{6,7}. This resembles mass defaecation flights of the giant Asian honeybee, *Apis dorsata*, which two of us (M.M. and T.D.S.) observed in Thailand in March 1984⁸. Although now realized to be common, such flights were previously unknown, perhaps because they occur at so great a height, even though hundreds of thousands of bees may be involved. Further, nearly all the Hmong to whom we showed bee faeces on leaves failed to identify them. Some said they were *kemi*, a Hmong term for the alleged chemical warfare agent.

Our conclusion that yellow rain is probably the faeces of honeybees and not a chemical warfare agent is thus supported by Hmong accounts of its appearance by pollen analysis, and by observations of the behaviour of honeybees in south-east Asia.

Studies of the yellow rain phenomenon in several countries, particularly in government laboratories, have still to be made public. It is to be hoped that the responsible officials will make every reasonable effort to move further investigation of this problem into the normal channels of scientific communication.

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Somatotopic map of the flying fox

SIR — In the interesting article by Calford *et al.* on the unusual somatotopic cortical map in the grey-headed flying fox, *Pteropus poliocephalus*, and also in the comment by Jones² on the article, one point escaped emphasis: the caudal shift of the somatotopic representation of the distal forelimb has taken place by reflection from rostral to caudal (that is the first digit or thumb remains lateral and adjacent to the face), instead of by rotation from rostral to caudal (in which event the thumb would lie medial and the fifth digit would be located adjacent to the face). In contrast to the reflection of the representation of the forelimb, the representation of the hindlimb appears to have rotated through about 90 degrees, so that it points medially (instead of rostrally as in most mammals). Whether this difference in the shift of the fore- and hindlimbs is related to their different functions (flying and feeding in the case of the forelimb, landing from flight in the case of the hindlimb) remains an open question.

Jones² has addressed a related issue: what is the advantage of representing the body surface on flat cortical maps instead of as a bolus of cells? Rostral/caudal and medial/lateral appear to be the spatial relationships in the periphery that need to be maintained in the cerebral cortex; dorsal/ventral seems a less important direction, being coded sometimes as caudal/rostral (for example in the axial/distal parts of a limb in quadrupeds) and sometimes as lateral/medial (for example in the position of the head relative to the limbs and trunk). If, then, one direction in the periphery does not have to be maintained in the cortex, a flat map can achieve all that a bolus would achieve, with the advantage of much less complex connections between thalamus and cortical surface.

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Spatial relationship and extrafoveal vision

SIR — A recent letter by Rentschler and Treutwein¹ describes a qualitative difference in human visual acuity between central foveal vision and a region 2° from the fovea when the task is to discriminate between stripe patterns that differ in appearance but are mathematically similar in everything but phase relationship between first and third spatial harmonics. For the pairs that are easily discriminated both foveally and at 2°, the appearance of the stripes differs in the contrast of a narrow dark band on a lighter grey stripe. In the patterns that can be discriminated foveally

and at 2°, the darker bands in each discrimination task are identical in quality and differ only in that their position with respect to the lighter bands is mirror-image reversed in the two images.

Rentschler and Treutwein conclude that (1) there is a qualitative difference between foveal and nonfoveal vision for spatial localization tasks but not for contrast detection tasks, and that (2) because of this difference between foveal and nonfoveal vision for one type of task but not the other, there must be two types of visual processing involved. We suggest that there might not be a qualitative difference between foveal and nonfoveal vision but rather a quantitative difference — spatial localization might simply decline faster than contrast detection as a function of eccentricity — and that this difference can be explained at least partly in terms of receptive field size and stimulus resolution in complex cells at various levels in the visual pathway, as a function of eccentricity.

Both the letter itself and the accompanying News and Views article by Watt² are couched in terms of the Fourier-analysis theory of cortical function; curiously no mention is made of known receptive field properties of cortical cells. As an alternative to their interpretation, which assumes that the early stages of vision can be regarded as a number of parallel spatial filters (a view that our receptive field studies do not seem to support), we would propose an interpretation (or perhaps a more physiological translation of their interpretation) in terms of the known properties of complex cortical cells. At a given eccentricity, the territory of visual field over which a complex cell can be activated is usually much larger than that of a simple cell, but the optimum stimulus size is approximately the same. A complex cell responds optimally to lines or slits in a specific orientation, but for an optimal response the line width is also critical. The optimal line evokes a response wherever it is shone over a wide area, even though a line thick enough to cover this entire area evokes no response at all. The optimal line width is roughly the same as the width of the region from which a simple cell can be activated, or the diameter of a retinal ganglion cell receptive field centre (for the same eccentricity), implying a preservation of acuity but loss of the spatial localization conveyed by simple cells.

This relative loss of what may correspond to spatial localization without loss of acuity is seen not only in complex cells in area 17, but even more strikingly in prestriate visual areas. Rovamo and Virsu³ have shown that human visual acuity at different eccentricities varies as a linear function of cortical magnification in area 17, and Rentschler and Treutwein therefore use magnification to scale the size of their two test patterns for eccentricity: they compare foveal and nonfoveal discrimination using only this one stimulus size ratio. This may be justified if one assumes that resolu-

tion (that is, acuity) as a function of eccentricity does not change as one goes further along in the visual pathway; physiological comparisons of areas 17 and 18 (see our paper, this issue⁴) support this assumption. But we think that magnification in area 17 might not be an appropriate scaling factor for tasks involving spatial localization. Spatial localization could well depend on the size of complex receptive fields, and that clearly does increase beyond 17, for example, in 18, V4 and MT, and also increases more rapidly for nonfoveal than for foveal vision.

The difficulty in discriminating mirror-image patterns is more likely to be related to spatial localization, and consequently tailoring stimulus size to cortical magnification in area 17 may be inappropriate. The mirror-image (spatial localization) discrimination is already poorer than the contrast discrimination in the fovea (Fig. 2 of ref. 1) and falls off more rapidly with eccentricity. This parallels the behaviour of complex cells at more central levels of the visual system than area 17. Thus the finding would seem to suggest not a qualitative difference in handling of form foveally and peripherally, but a difference in rates of deterioration with eccentricity between acuity as commonly defined, and something else, possibly spatial localization.

We further suggest that these results do not necessarily implicate two separate visual processes for contrast discrimination and spatial localization. The fact that the relative loss of spatial information seen physiologically in single cells is paralleled in these psychophysical observations suggests to us that spatial localization and contrast information are probably encoded in the same cells and not by separate processes. Only if psychophysical spatial localization were better than predicted by the physiology of complex cells would one need to invoke a separate process for it.

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Lysosomes and prohormone activation — correction

IN the letter from C. N. Hales under this title in *Nature* of 7 March (Vol. 314, 20) an editorial change altered the sense of the final paragraph which should read:

The sequence information which has emerged to date is consistent with the lysosomal hypothesis, suggesting that it may provide a useful framework for making and interpreting structural comparisons in this area.

The hypothesis referred to is outlined in the second paragraph of the letter and in more detail in the ref 3 and 4 in the list cited.

How many orbiting lasers for boost-phase intercept?

from Richard L. Garwin

A constellation of laser battle stations in low Earth orbit could intercept intercontinental ballistic missiles (ICBMs) in their boost phase. If such lasers are available decades hence, they will face fast-burn ICBMs and find their task more difficult. How difficult is shown by an elementary analysis taking into account laser retarget time.

PRESIDENT Reagan's Strategic Defense Initiative (SDI) for countering Soviet nuclear-armed intercontinental-range ballistic missiles (ICBMs) and submarine-launched ballistic missiles (SLBMs) puts great stress on interception of the missiles in boost phase. Although the feasibility of a star wars defence will stand or fall by the ease of countering the system, it is of some interest to know how many satellites of given characteristics would be required to destroy boosters in the numbers and with the characteristics that may be available by the time such a defence could be mounted. (See ref. 1 for a technical introduction to boost-phase intercept.)

The emphasis given in this discussion to the need to destroy most ICBMs while the rocket booster is still burning is consistent with the general supposition by SDI supporters that this would be essential even to partial defence, because the missile is then more visible and more vulnerable than later, when the reentry vehicles and any decoys may have been deployed; moreover, in the boost phase there are fewer targets to be destroyed.

The Soviet silos lie deep within the Soviet Union, and two basing modes have been suggested for boost-phase interception. The first entails large numbers of low-orbit satellites carrying powerful lasers or homing rockets, so that there will always be enough within range of any silo to destroy the ICBM launched therefrom. The second involves pop-up interceptors based on submarines near the Soviet Union, that would be launched on information that a massive ICBM launch had taken place and which would destroy missiles in boost phase with X-ray lasers powered by nuclear explosions. If the X-ray laser is assumed feasible and effective, the need to bring it above the curve of the Earth during the duration of boost of the ICBM requires an interceptor mass far larger than the payload mass itself. Thus² an interceptor based 7,400 km from the silo, required to climb in 120 seconds to an altitude giving a line of sight with 185-km Earth-limb clearance to a booster which has climbed to 370 km altitude, would have an initial mass of 660 tons to lift an X-ray laser payload of 1 ton. This assumes no structural mass needed for the

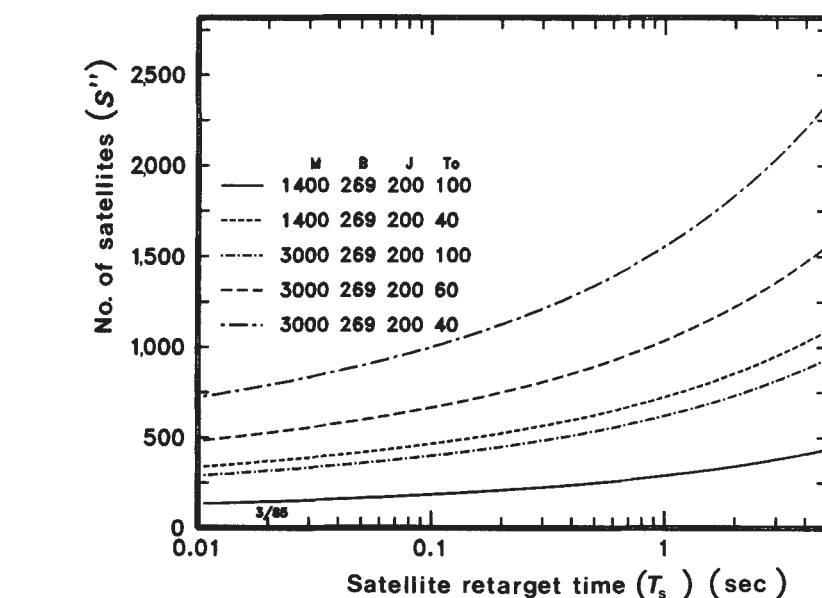


Fig. 1 The number S'' of our standard laser satellites is plotted against assumed laser retarget time for two assumed booster numbers and 3 assumed engagement times during boost phase. Against the clustered launch of 3,000 boosters of 40-s engagement time, 1,344 satellites of 0.5-s retarget time in optimum orbit are required, working perfectly reliably, to dispatch the boosters.

rocket, a specific impulse of 300 seconds (exhaust velocity of 3 km s^{-1}) and instantaneous burn of the interceptor rocket propellant. To reach the same altitude in 60 seconds would require twice the speed and an initial mass of 430,000 tons. These are some of the reasons why what follows concentrates on the characteristics of the first basing mode for SDI, a satellite constellation.

Counter measures

In what follows, I consider the likelihood that there will emerge counters to the assumed defences, for example, by the evolution of the Soviet ICBM force to small single-warhead missiles equipped with fast-burn boosters. Such missiles, perhaps burning out in 50 seconds at an altitude of 80 km, would negate the pop-up interceptor in two ways: first, it could not climb to line-of-sight within 50 seconds, while the soft X-rays could not penetrate to 80-km altitude within the atmosphere. Studies made for the US

government in July 1983, by McDonnell-Douglas and Martin-Marietta, indicate that such fast-burn boosters with MIRVs and penetration aids could be built at a cost of only some 10–15 per cent more than optimized (normal-burn) boosters. Indeed, given that the cost of the missile guidance systems may be much reduced, a silo-based single-warhead ICBM could even be cheaper per warhead than a new MIRVed large missile because of economies of scale in production, and the much cheaper development and test programme.

Missile defence based on satellites in low Earth orbit would provide continuous visibility from some satellite to each booster, but fast-burn missiles would again negate the capability of both X-ray lasers and neutral particle beams (hydrogen-atom beams of 100-MeV energy) because of the screening of the atmosphere. Homing interceptors would have little time to reach the boosters and the atmosphere at 80-km altitude would

interfere with their infrared homing. Satellites carrying powerful continuous-wave or repetitively pulsed infrared lasers can, at some wavelengths, see down to the clouds and might have significant capability against individual missiles, even fast-burn boosters. We assume that a 50-second-burn booster could be engaged for 40 seconds by such a satellite fleet, but note that such large satellites would need to be protected against Soviet antisatellite weapons.

There are few published analyses of required numbers of laser battle stations, and these are examples of methodology rather than conclusions, since they neglect the relationship between a satellite's vulnerability and its altitude, assume zero retarget time, and make other simplifying assumptions. Carter³ chooses to deploy his satellites on 1,000-km altitude orbits and, for booster hardness half that assumed here, estimates 160 satellites required to handle the present 1,400 Soviet boosters as they are now distributed. For the same boosters deployed "in one region", Carter estimates that 500 satellites are required.

Other estimates⁴ point to a requirement of 300 lasers, or 800 lasers (the latter estimate involving satellites at 1,000-km altitude deployed in orbits of optimum inclination and assumed to destroy a booster out to 3,000-km range in the 7 seconds required at 3,000 km). The assumption that satellites are deployed for uniform coverage of the entire globe⁵ leads to a requirement for some 2,400 satellites⁶.

In unpublished Los Alamos documents of 4 May and 6 August 1984, G. H. Canavan⁷ assumes satellites distributed uniformly over the entire globe and asserts that for boosters uniformly distributed over a very large region, satellite numbers required grow only as the square root of booster numbers (or hardness) because satellites uniformly deployed will be closer together and will destroy boosters at shorter average range. These results are cited in ref. 8. For deployment in a small region, Canavan also obtains satellite numbers proportional to the number of boosters.

C. T. Cunningham⁹ assumes 300-km orbit altitude, with 60-deg. orbits; using a dynamic simulation, he finds that only 60 satellites are required—noting however that his simulation requires "that the switching time be much less than the dwell time of 42 milliseconds at the minimum target distance of 100 km."

The present analysis explicitly considers retarget time for both distributed and regional basing of ICBMs. We consider a constellation of satellites assumed to provide, over the region of ICBM silos, an areal density s (number of satellites per unit area) adequate to destroy all ICBMs, assumed launched simultaneously and burning out in time T_0 .

The derivation of the overall number of satellites S from the required local areal

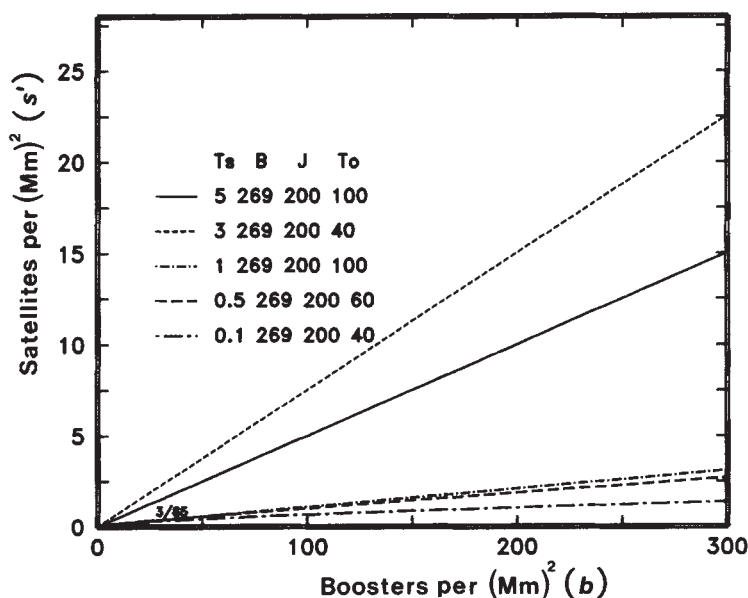


Fig. 2 Perfect satellites as in Fig. 1 are uniformly distributed at optimum (near-zero) altitude above a distributed booster density of b and are constrained to shoot within their tiling hexagons. Soviet missiles are said^{3,4} to number 1,400 and be distributed over an area of 10 (Mm)^2 ; thus $b = 140$. With a 5-sec laser retarget time and 100-sec engagement time, some 5.8 satellites per $(\text{Mm})^2$ are needed—or 950 in optimum orbit world-wide.

density s entails the calculation of s as a function of orbit parameters. Satellites in a mixture of orbits giving uniform density everywhere will occupy $4\pi E^2 = 509(\text{Mm})^2$ of area, so that $S = 509s$. Launch sites clustered at the North Pole could be attacked most effectively with polar-orbit satellites, which have a much larger latitude-band-averaged density at $\lambda = \pm 90^\circ$ than elsewhere. Silos limited to the Equator could be efficiently addressed by equatorial low-Earth-orbiting satellites. In general, there will be an optimum orbit to attack boosters launched from silos spread uniformly from latitudes λ_1 to λ_2 . If we assume satellites uniformly phased in orbits of optimum inclination i , the band-averaged satellite density

$$\langle n \rangle_{\lambda_1, \lambda_2} = \frac{\int_{\lambda_1}^{\lambda_2} n(\lambda) \cos \lambda \, d\lambda}{\int_{\lambda_1}^{\lambda_2} \cos \lambda \, d\lambda} \quad (1)$$

where $n(\lambda)$ is the satellite density averaged by Earth rotation for latitude λ . This is an optimistic measure. For satellites of very short range, the constellation capability is limited by $\min(n(\lambda))$ over $\lambda_1 < \lambda < \lambda_2$. For long-range satellites, those outside the latitude range will have to be considered, which will reduce the average density below that calculated here, so that the concentration factor y derived here is an over-estimate, especially because the granular satellite density is assumed uniform.

For N satellites on any orbits of inclination i , the longitude-averaged

$$n(\lambda) = (N/2\pi^2)/(\sin^2 i - \sin^2 \lambda)^{1/2} \quad (2)$$

for $\lambda < i$, and $n(\lambda) = 0$ for $\lambda > i$. Averaging over a band from λ_1 to λ_2 , we have for

such orbits

$$\langle n \rangle_{(\lambda_1, \lambda_2)} = (N/2\pi^2) \times \frac{[\sin^{-1}(\sin \lambda_2 / \sin i) - \sin^{-1}(\sin \lambda_1 / \sin i)]}{(\sin \lambda_2 - \sin \lambda_1)^{-1}} \quad (3)$$

(for $\lambda_1 < \lambda_2 < i$, and with obvious modifications for $\lambda_2 > i$). For silos spread uniformly between 50° and 60° , the maximum concentration factor y is reached with orbital inclination $i = 60^\circ$, and is $y = 3.1$, which we have used in constructing Table 1 and Fig. 1. Thus we multiply required satellite densities by 164 (Mm)^2 , confident that the resulting number of satellites will always be an underestimate.

We assume a steady or rapidly pulsed laser on each satellite, provided with an optically perfect diffraction-limited mirror of diameter D which is refocused at the range of that booster which is the target of the moment. (The Hubble space telescope will have $D = 2.4 \text{ m}$; demonstration of even megawatt diffraction-limited chemical lasers is several years away in the SDI programme.) The laser is assumed to have a steady output power P and wavelength λ . For a uniformly illuminated output aperture of area $A = \pi D^2/4$, the maximum possible power density at any point in the focal spot at range r is obtained from the brightness B of the laser (power per steradian of solid angle) as $p = B/r^2$. In turn, B is given precisely by $B = PA/\lambda^2$, which can be shown by integrating over the radiating aperture to obtain the electric field a short distance away, and comparing with the electric field when the entire aperture is phased to provide a focus at distance r .

In the numerical examples we consider $P = 25$ MW, $D = 10$ m, $\lambda = 2.7$ micrometre (hydrogen fluoride). We thus take $B = 2.69 \times 10^{20}$ watts per steradian. At $r = 3,000$ km or 3 Mm, the power density is thus 30 MW m^{-2} . We assume a booster hardness $J = 200 \text{ MJ m}^{-2}$, which is sufficient to evaporate a layer of carbon 2 mm thick. Then the time $t(r) = Jr^2/B$ required to deliver the assumed lethal heat is 6.7 seconds. Such calculations assume that the satellites can see the boosters sufficiently clearly and can point sufficiently accurately and stably that the power density at the peak within the 1-m diameter spot (at 3-Mm range) is not diminished by spot wander exceeding a few tens of cm during the 40 km or so travel of the booster during the 7-second engagement.

As one considers countering missiles with boost times ranging from the 300 seconds or so of the Soviet SS-18 to the 180 seconds of the US MX missile and to the 40 seconds of a fast-burn ICBM, the need arises either for more satellites or for satellites at closer range. Furthermore, particularly for powerful satellites or for close-range engagements, the time T_s required for the laser satellite to retarget, to slew and settle the beam to the necessary accuracy of 0.1 microradian or better, plays an important role in determining the size of the constellation.

Our programme is first to calculate, for a flat Earth and a planar satellite constellation at altitude h , the number of missiles launched from a region that could be killed by a constellation of satellites with particular density and laser characteristics. We then extend that calculation to the more realistic case of a spherical Earth, obtaining a reasonably accurate closed-form expression (Fig. 1). Next we calculate the missile-killing potential of an assumed satellite constellation density above a uniform areal distribution of boosters, for which each satellite is assigned the boosters within its circle of responsibility (Fig. 2). This would be a reasonable assignment strategy for boosters covering the entire Earth at uniform density, but it has also been used to justify "square-root scaling" of satellite numbers against booster numbers for boosters deployed over $10 (\text{Mm})^2$, taken to be the deployment area of the 1,400 current Soviet ICBMs^{7,8}. The results are tabulated in Table 1 and discussed for some cases of interest.

Although there seem to be no published results on laser constellation size for finite retarget time, our calculations show the great importance of the time required to move the beam from one booster to the next.

Clustered launch

We now turn to the problem of relating local satellite density to the number of boosters to be destroyed. For M_0 boosters all launched simultaneously from a point,

Table 1 Required satellite densities s , and numbers S .

M boosters	J (MJ m ⁻²)	T_0 (s)	T_s (s)	s' (per (Mm) ²)	S'	s'' (per (Mm) ²)	S''
1,400	200	100	3	4.24	697	2.32	381
1,400	200	40	3	10.54	1,732	5.80	953
3,000	200	40	3	22.5	3,699	12.43	2,044
1,400	200	100	0.5	0.89	146	1.53	251
1,400	200	40	0.5	1.96	322	3.82	628
3,000	200	40	0.5	3.97	653	8.18	1,344
3,000	200	100	0.5	1.71	281	3.27	537
1,400	200	100	0.1	0.48	79	1.14	187
1,400	200	40	0.1	0.84	138	2.85	468
3,000	200	100	0.1	0.76	126	2.44	401
3,000	200	40	0.1	1.39	228	6.10	1,003

Boosters distributed over $10 (\text{Mm})^2$ (solution of Eqn. 18):
(Lasers assigned local areas of responsibility)
(Flat Earth, satellite numbers will be greater than shown)

Concentrated boosters with horizon cutoff of satellite effectiveness:
(Round Earth, solution of Eqn. 13, optimum altitude $h^2 = (BT_s/J)$)
For $T_s = 3, 0.5, 0.1$ seconds, h is 2,010, 820, or 370 km, respectively.

The point-launch approximation holds for $G \leq h$, where G is the diameter of the launch field.

Orbiting satellites carrying 25-MW lasers of 2.7 μm wavelength and with 10-m diameter perfect mirrors (brightness $B = 2.69 \times 10^{20}$ W per steradian) are placed in near-optimum 60° inclination orbits for boost-phase intercept of ICBMs near 55° latitude. The last two columns present the local satellite density s'' to destroy the indicated number of missiles of boost time (engagement time) T_0 , with satellites of retarget time T_s , assuming the missiles concentrated in a region not much bigger than the altitude of the satellites. Total number S'' of satellites needed is obtained by multiplying s'' by the surface area of the Earth— $509 (\text{Mm})^2$ —divided by a concentration factor of 3.1. For this concentrated launch, optimum orbit altitude (Eq. 12) is 2,010, 820, or 370 km respectively for retarget times of 3, 0.5, or 0.1 s. Columns s' and S' are an improved analysis of an assumed distribution of boosters clearly inapplicable to the parameters discussed here—as if boosters were deployed at constant density everywhere, with satellites constrained not to shoot at a booster if there is a satellite closer to that booster. This model would clearly require more satellites than if an optimum assignment were used, as in calculating the density s'' against clustered launch. Equally clearly and primitively, distributed boosters should be an easier task for the defence—a correct intuition contradicted by the comparison of 697 satellites and 381 satellites in the first row of the table. The discrepancy arises because the assumed model^{7,8} is inappropriate.

and if a satellite constellation of local density s and brightness B , deployed at altitude h , is assigned to kill them, we assume a continuous distribution of satellites and that the beam is always normal to the booster surface. Since the time to kill a booster at distance r is Jr^2/B , a satellite at distance r can kill $m(r) = (BT_0/Jr^2)$ boosters in the available boost time T_0 .

The missile-killing potential of the constellation against clustered launch is then obtained by integrating $m(r)$ over all satellites from those directly above the launch site ($r = h$) to some maximum r . We cut off the integral at $r = U$, defined as the distance beyond which a satellite can no longer kill even one booster. Then U is given by $m(U) = 1$, or $U^2 = BT_0/J$. This calculation optimistically takes credit for fractional kills above unity. Each satellite closer than U is assigned a different booster and heats it for the required time before being assigned and moving instantly to heat another. The number of boosters that can be killed, M , is then

$$M = \int sm(r) 2\pi r dr = (BT_0/J) \int_{\rho=0}^{\rho=\max} \frac{2\pi s \rho d\rho}{(h^2 + \rho^2)} \quad (4)$$

where the coordinate system is fixed at the booster launch point, the integration runs over the various satellites and ρ is the radius from the origin to a sub-satellite point. With $a \equiv r^2 = h^2 + \rho^2$,

$$M = \pi s (BT_0/J) \int_{a=h^2}^{a=U^2} da/a = \pi s (BT_0/J) \ln (U^2/h^2) \quad (5)$$

$$= \pi s (BT_0/J) \ln (BT_0/Jh^2) \quad (6)$$

For the 25-Mw 10-m diameter HF laser described earlier, we have $B = 269 \text{ Mw(Mm)}^2 \text{ m}^{-2}$, $J = 200 \text{ MJ m}^{-2}$ and, for $T_0 = 100$ s, $U = 11.6$ Mm. For $h = 500$ km (0.5 Mm), the logarithm is about 6 and varies only slowly with h .

A planar constellation of one satellite per square megametre and zero retarget time, consisting of some 164 satellites in optimum orbit covering only Soviet ICBM sites, could thus destroy 2,657 missiles of the assumed characteristics. But, as will be seen, the recognition of satellite retarget time of 0.1, 0.5, or 3.0 seconds reduces this capability to 2,475, 2,107 and 1,468 boosters respectively. The imposition of horizon limitations for the case of a curved Earth will then further reduce the capability of the constellation.

Flat Earth and non-zero retarget time. A 10-m diameter mirror cannot move instantly from one target to another. The Hubble space telescope has a settling time of 3 seconds for the smallest motion within a beam-width (about $0.2 \mu\text{radian}$), and will take several minutes to move to a new target. Because of the large laser power at the mirrors, it is not possible to make use of tricks often used in optical systems for compensating imperfect figure or orientation of large elements by the deformation or motion of smaller elements. For the same reason, and because the beam must be moved typically 6° or so between targets (some 4×10^5 beamwidths) electro-optical phase-shifting coatings cannot do the job. It might be possible to retarget a 10-m mirror to within 0.1 microradian or less in, say, $T_s = 3$ seconds. We shall consider $T_s = 3, 0.5$, and 0.1 s. Kill time Jr^2/B then becomes $T_s + Jr^2/B$, which is equivalent to replacing r^2 by $(r^2 + BT_s/J)$ in the calculation of M . We then have

$$M = \pi s (BT_0/J) \ln \left[\frac{(U^2 + BT_s/J)}{(h^2 + BT_s/J)} \right] \quad (7)$$

Round Earth. The horizon (tangent plane to the Earth at the booster launch location) intersects the satellite constellation sphere at a distance

$$H = [(E + h)^2 - E^2]^{1/2} \quad (8)$$

Satellites beyond that distance are below the horizon and shielded from the booster at low altitude. For example, $H = 1.94$ Mm for $h = 300$ km, far less than the upper limit of the integral for calculating M in the flat-Earth case, for which U exceeds 11 Mm. Apart from small effects relating to the somewhat reduced range to a satellite on the basing sphere at ρ , the spherical distribution of satellites can be taken into account. With

$$U' \equiv \min[(U), (H)] \quad (9)$$

Equation (7) becomes

$$M = (\pi s BT_0/J) \ln \left[\frac{(U'^2 + BT_s/J)}{(h^2 + BT_s/J)} \right] \quad (10)$$

or, for the normal horizon-limited case,

$$M = (\pi s BT_0/J) \times \ln [1 + 2hE/(h^2 + BT_s/J)] \quad (11)$$

In order to use the satellites to best advantage, we maximize M with respect to h . The logarithm is maximum when the argument is maximum, which for the horizon-limited case, yields the criterion

$$h^2 = BT_s/J, \quad (12)$$

For this optimum altitude, we then have

$$M = (\pi s BT_0/J) \times \ln [1 + E(BT_s/J)^{-1/2}] \quad (13)$$

valid so long as $H^2 < U^2$, or so long as $2E(BT_s/J)^{1/2} + (BT_s/J) < U^2$. For $B = 269$, $J = 200$, this condition is satisfied up to $T_s = 9.8$ seconds for $T_0 = 40$ and $T_s = 17.5$ s for $T_0 = 100$. The results for the numbers of boosters killed are plotted in Fig. 1.

Distributed boosters

We now turn to the question of the validity of the claimed "square-root scaling" of satellite density with increasing booster density. To calculate the constellation densities to destroy boosters distributed uniformly (with density b) over a plane, we assume satellites on a regular triangular (hexagonal close-packed) lattice at altitude h , with nearest-neighbour satellite spacing $2R$. To each satellite is assigned the task of destroying every booster in its own circle of responsibility of radius R (which strictly is a hexagon whose area is 10.3 per cent greater than that of the intended circle). The capability of each satellite is exhausted when the sum of the times taken to kill each booster (including retarget time) exceeds the available engagement time T_0 . For a given booster density b , we calculate the time T required for a satellite to kill its "own" boosters, which is a function of the parameters b , J , h , R , B and T_s . If the laser parameters B and T_s are fixed, we maximize constellation capability with respect to h for a given satellite density $s \equiv 1/\pi R^2$ and thus determine b , which gives a point on the b - s curve of booster density against satellite density (or number). If $t(r)$ is the time to kill a booster at distance r , and ρ is now the radial coordinate on the ground, with origin at the sub-satellite point, the time

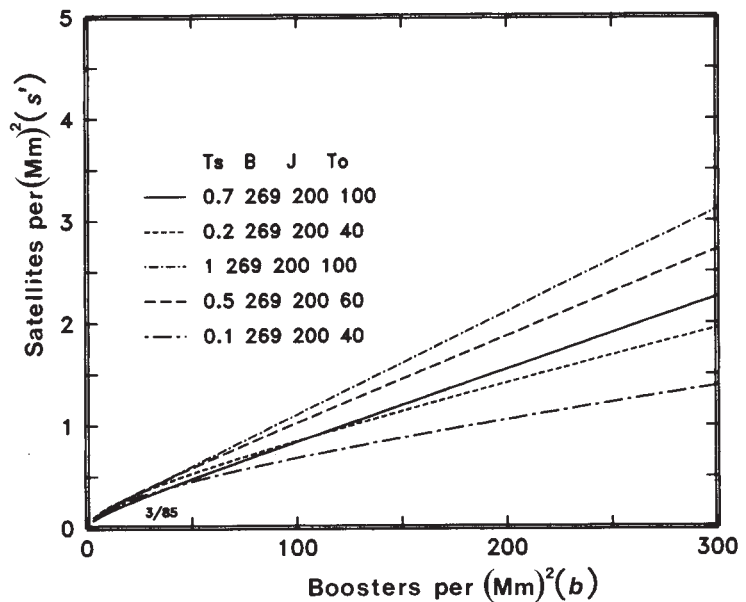


Fig. 3 Shorter retarget times T_s than in Fig. 2 are modelled here. Note that the "square-root scaling"^{3,4} is confined to low booster density and is replaced by a linear relationship at high booster density. Satellite density s' exceeds the greater of bT_s/T_0 and $(Jb/2\pi BT_0)^{1/2}$. Thus a model which is inapplicable because of geographic distribution also fails to give favourable square-root scaling at large threats when finite retarget time is considered.

required to kill all boosters is

$$T \equiv \int_{\rho=0}^{\rho=R} t(r) b 2\pi \rho d\rho \quad (14)$$

The integral extends over all the boosters within radius R of an individual satellite. With $t(r) = T_s + Jr^2/B$ and $a \equiv r^2 + (BT_s/J)$, the time T becomes $\pi Jb/B \int a da$ between limits which are the extreme values of a (differing by R^2), which leads to the result

$$T_{\min(h)} = (2\pi Jb/B)(R^4/4) \times [1 + (2/R^2)(BT_s/J + h^2)] \quad (15)$$

This time is a minimum at $h = 0$, whence

$$T_{\min(h)} = (2\pi Jb/B)(R^4/4) \times (1 + 2BT_s/JR^2) \quad (16)$$

and

$$T_0 = (Jb/B)(\pi R^2)^2(2/4\pi) \times (1 + 2BT_s/JR^2) \quad (17)$$

$$s^2 = (Jb/BT_0)(1/2\pi) \times [1 + (2\pi T_s B/J)s] \quad (18)$$

Satellites cannot be in orbit at zero altitude, so that s defined by this quadratic equation will again be an underestimate. The equation determines the satellite density s required to cope with an infinitely extended booster distribution of density b .

A more flexible assignment of targets would require fewer lasers, but we take our restrictive assignment scheme as a prerequisite (but not a sufficient condition) for a claimed square-root scaling, in order

to explore the actual scaling law. The solution of the equation is

$$s = (T_s/T_0)(b/2) \times \{1 + [1 + (1/b)(2/\pi)(T_0 J/BT_s^2)]^{1/2}\} \quad (19)$$

If $b \gg b^* \equiv (2/\pi)(T_0/T_s)(J/BT_s)$, then $s \approx bT_s/T_0$ and is linear in booster density. For the parameters assumed, our $b^* = 5.3$ per (Mm)² is far below the existing 140 per (Mm)² density of Soviet ICBMs, and the number of satellites required is linear in the number of boosters deployed. In terms of satellite density, $(2\pi T_s B/J)$ is 25.36 for satellite retarget time $T_s = 3$ seconds. Thus the second term in equation (18) dominates the first for $s > 0.04$ satellites per square megametre. Much weaker satellites, or shorter retarget time, or tougher boosters will be required to obtain "square-root scaling," even for infinite distributions of boosters and satellites^{7,8}.

Equations (18) and (19) teach us that "square-root scaling" can enter only at low satellite density or low booster density, respectively. But a stronger inference can be drawn from Equation (19) regarding the scaling of numbers of satellites of given characteristics versus numbers of boosters of infinite extent. Clearly, the asymptotic behaviour for large numbers of boosters is $s = bT_s/T_0$ —each local satellite destroying a booster each retarget time, for the entire burn or engagement time. But Equation (19) tells us that satellite numbers are always greater than that given by the asymptotic formula, and in the linear range this residue of square-root scaling requires an additional satellite density $s_+ = J/2\pi BT_s$. Thus we know that even on the assumption of widely deployed boosters, the required local density of satellites is at least the local density of boosters multiplied by T_s/T_0 , and the ratio of the cost of the satellite constellation to the cost of the booster constellation exceeds

$$(C_s/C_b) \times (T_s/T_0) \times (4\pi E^2/\gamma A) \quad (20)$$

where C_s is the cost of a satellite, C_b the cost of a booster, and A the area over which the boosters are deployed. Adopting the proposed^{7,8} assignment strategy that satellites should be restricted to shoot within their circles of responsibility (as required for square-root scaling), and assuming the deployment area to be 10

square megametres and a satellite concentration factor $\gamma = 3.1$ from optimum orbits, satellite numbers are $16T_s/T_0$ times the number of boosters. With 3-second retarget time and 100-second engagement time, one satellite must be deployed for every two boosters, and the defense would be overcome by a cheaper offensive deployment if a booster cost less than half the price of a laser battle station.

Since the laser must have been launched into orbit by a rocket, and weighs far more than the ICBM payload, and because lasers must be deployed over an area very much larger than the booster deployment area (to which active lasers are limited by the assignment strategy needed for square-root scaling), boost-phase intercept can hardly be achieved cost-effectively by satellites armed with powerful lasers. Furthermore, for non-zero retarget time and for reasonable satellite and booster characteristics, satellite numbers are proportional to booster density even for infinitely extended booster deployment. Because satellites are assumed to shoot only at local boosters in the distributed launch case, there appears to be little difference between the planar constellation and round-Earth results for distributed launch, although the smaller values of S' in Table 1 would be increased by recognition of horizon limitations which impose $h > 0$.

With the simplistic assignment of satellites to kill widely distributed boosters in their immediate vicinity, Equation (18) shows a number of satellites linear in booster density except for near-zero retarget time, and then only for satellite altitude zero or scaled with satellite separation, and also only for low booster densities. According to Equation (19), large booster density, short burn time, reduced booster hardness, enforce linear scaling; while poor laser brightness and short retarget time are conducive to square-root scaling. Figure 2 and the columns in Table 1 labelled s'' and S'' demonstrate near-linear scaling for many cases of interest with distributed boosters.

This rather primitive assignment of satellite resources is in fact far from optimum in some of the cases considered here, and since a defensive system will be two decades or more in evolution, it is realistic to consider the satellite numbers required to overcome a more responsive

threat—the clustered launch of smaller missiles.

The last two columns in Table 1 present the satellite density and numbers required to handle the indicated booster threat for launch of boosters from an area some hundreds of km diameter. We assume 25 MW of 2.7 micrometre laser light; 10-m diameter perfect optics; retarget time T_s of 0.1, 0.5, or 3.0 seconds as indicated; engagement duration T_0 . For concentrated launch, the required number of satellites is proportional to the number of boosters.

Figure 3 demonstrates that only for retarget time of 0.1 second is there such a thing as "square-root scaling" at existing booster numbers. Furthermore, Table 1 and Fig. 1 illustrate that the Soviets have a far more effective response than to build additional silos distributed over their vast territory—namely to cluster them in a region 1,000-km diameter, or so, and to pay the modest additional cost to shorten burn time to 40–50 seconds. This would also negate boost-phase intercept by satellites carrying X-ray lasers, particle beams, and by any pop-up weapon. Such a response would ensure that boost-phase intercept could not meet the requirement stated for the US Government on 20 February by P. H. Nitze that it "be cheap enough to add additional defensive capability so that the other side has no incentive to add additional offensive capability to overcome the defence."

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Nuclear collisions at intermediate energies: achievements and prospects of GANIL

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At energies of tens of mega-electronvolts per atomic mass unit, nuclear collisions exhibit phenomena more complex than the essentially collective behaviour at lower energies or the more individualistic nucleon interactions observed at much higher energies. This intermediate regime is being uniquely explored at the French national heavy-ion accelerator GANIL.

THE French facility for heavy-ion physics, GANIL (Grand Accélérateur National d'Ions Lourds), has been operating since 13 January 1983. The first beam from this accelerator, which uses a series of three cyclotrons to bring the velocity of ionized atoms to about one third of the velocity of light, had been obtained two months earlier, in November 1982. From the start, GANIL was thought of as a national facility. This had two consequences: first, the formation of a very able team of engineers and technicians from all French laboratories; second, the choice of an accelerator that would represent a significant improvement on anything previously built in France, but which was within reach.

The nature of the beams to be accelerated and the type of the accelerator were decided in 1972–74. At that time, heavy-ion research had just established itself in the study of atomic nuclei. For some years, many physicists had considered it to be a backwards step from the precise and successful analysis of discreet nuclear states that the accelerators of light ions, such as protons and α -particles, had provided. Massive projectiles, such as the ions of carbon, calcium and heavier elements, with poor energy resolution, were described by some as awkward tools for the study of the subtle quantum structure of nuclei—not much better, perhaps, than the use of boxing gloves to put thread through the eye of a needle. Yet, in about 1970, it was clear that nucleus–nucleus collisions, as made possible by heavy-ion beams, were unique means of studying the collective coherent behaviour of nuclei. New phenomena were discovered and the lexicon of nuclear physicists had to be expanded to include new phrases such as dissipative phenomena, temperature, time evolution and friction, which undoubtedly reflected increasing knowledge about nuclei. French groups had been particularly active in this field, with a major part being played by the ALICE accelerator in Orsay.

What was the next step? One problem arose from the mismatch between the turnover time for an experiment, typically 6 months to 2 years, and the time needed to design and build a heavy-ion accelerator, typically 5–10 years (constructing an accelerator for particle physics takes about the same time but experiments last several years. At the other extreme, in most fields of physics, the turnover time of experiments is measured in months, as in nuclear physics, but the equipment is usually lighter and can

be transformed at a similar rate). Hence, at the beginning of the 1970s, French nuclear physicists had to design an accelerator for the 1980s and 90s while hoping that the next experiments would help to elucidate the mechanisms of dissipation of excited nuclear systems within the next year. But choices had to be made, and three orientations were agreed upon: (1) The new accelerator had to accelerate ions up to velocities of 100 MeV AMU^{-1} with no provision for extending that range further. (2) The structure of the accelerator would consist of a small compact injector cyclotron followed by two large separated-sector cyclotrons operating in series (Fig. 1). Other possibilities were discarded. A linear accelerator would have opened up a regime field not very different from that well explored by GSI Darmstadt. Cryogenic cyclotrons would have proved difficult to design and operate safely, a risk that a relatively small community could not afford. The coupling of an ion source of high ionization ability with a synchrotron would, it was felt, limit the intensity of the beams and rely too heavily on the overcoming of technical problems not then solved. The choice made seemed to represent the best compromise between innovation and security. (3) The new laboratory had to be a national facility, therefore all agencies and laboratories had to contribute in manpower and financially to ensure the successful completion of the GANIL programme.

Intermediate energy physics

Next, I discuss the reasons that determined the choice of the beam energies available at GANIL. The idea was to study nuclear collisions in an energy range where a transition between two regimes occurs. Figure 2 shows the domain available with GANIL in comparison with other existing or projected accelerators. At energies of several tens of MeV AMU^{-1} the processes at work when two nuclei collide should change markedly. I will address four such arguments.

First, the wavelength λ associated with a nucleon of a projectile with an energy of say 5–10 MeV AMU^{-1} , available with major cyclotrons and tandems, is much larger than the mean distance d between two nucleons of the target nucleus. Thus, the incoming nucleon will 'see' (that is, interact with) several target nucleons. The collision process will then seem to be essentially collective. In the 1960s and 70s one could thus study the fusion process in which the two colliding nuclei fuse into a single excited nuclear system, and the so-called deep inelastic collisions where a temporary binuclear system is formed before the two partners separate again. The latter, an unexpected process, offered significant new information on the collective properties of nuclear matter and even, at a more elementary level, on the mechanisms that allow the propagation and dissipation of energy and angular momentum.

At the other extreme, say at 1,000 MeV AMU^{-1} energy, the situation is clearly the opposite, λ becoming smaller than d . The reaction mechanism results from the superposition of nucleon–nucleon interactions. The degree of overlap of the two colliding nuclei determines the dominant behaviour. In central collisions complete explosion is observed, whereas in peripheral ones a

Some 30 articles dealing with experimental work done at GANIL have been published so far. They are systematically compiled and discussed in *Nouvelles du GANIL*, published bimonthly and sent on request to all interested scientific institutions.

Among the general papers (unpublished) dealing with GANIL nuclear physics are:

M. Lefort *Lectures to the Cargèse Summer School 'Heavy-Ion Collisions from Nuclear Collective Motion to Quarks'* (September 1984; preprint IPN-Orsay IPNO-DRE-84-33).

B. Tamain *Lecture to the 16th International School on Nuclear Physics* (Mikolajki 1984; preprint LPC Caen 84-03).

C. Detraz *Mt Fuji Symposium on Heavy-Ion Physics 'First results from GANIL'* (Tokyo, August 1984).

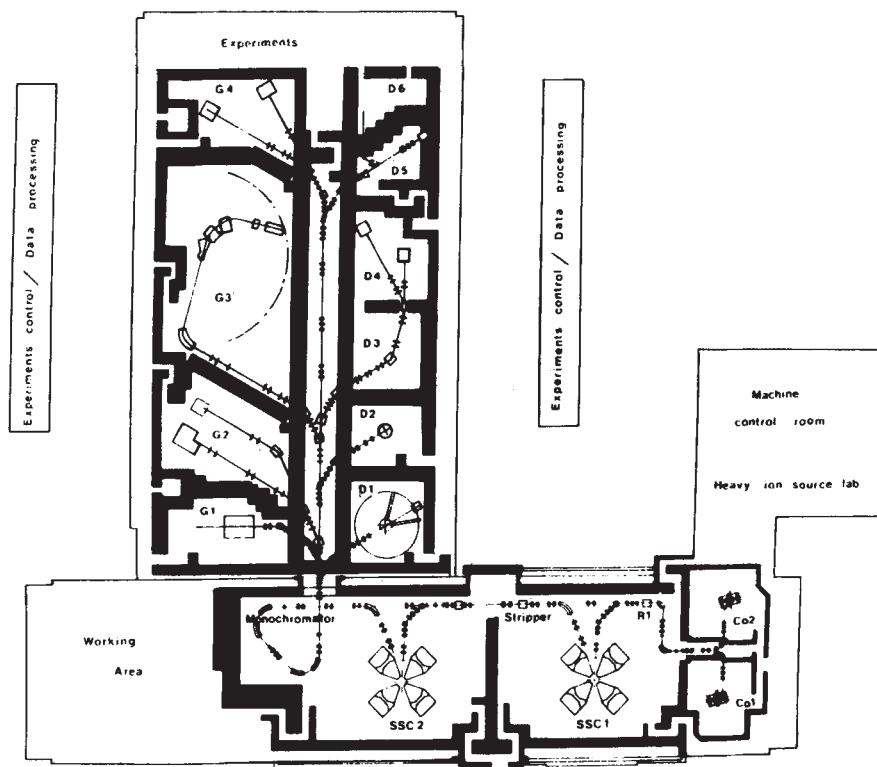


Fig. 1 Layout of the GANIL accelerating system, consisting of one compact cyclotron injector and two large separated-sector cyclotrons (SSC). A thin carbon-foil stripper increases the charge stage of the accelerated ion before entering the second SSC. An α -shaped magnetic analyser selects the momentum of the accelerated particles before the beam is transported to the various experimental areas.

participant zone, highly excited and emitting many fast nucleons, can be distinguished from the spectator zones corresponding to the non-overlapping parts of the nuclei. Despite their very low beam intensity, those high-energy machines that could accommodate heavy ions, such as the Bevalac in Berkeley and the synchrophasotron in Dubna, confirmed this overall picture.

Second, the quantum nature of the two colliding nuclei means that the interaction of two of their nucleons, which are fermions, is only possible if they can be scattered into unoccupied discrete states. Because all the quantum states below the Fermi level are occupied in both nuclei, this requires that the energy available in the nucleon-nucleon collisions be large enough to promote these nucleons into highly excited states. In terms of velocity instead of energy, individual nucleon-nucleon interactions play a significant part in the nuclear collision only if the relative velocity of the nucleons is larger than the Fermi velocity, which is equivalent to $\sim 35 \text{ MeV AMU}^{-1}$.

Third, the velocity of sound in nuclei can be calculated from what is already known about the elementary modes of excitation of nuclear matter. An elementary local perturbation of the wave function of nucleons propagates with a velocity of $\sim 30 \text{ MeV AMU}^{-1}$. When the relative velocity of colliding nucleons is far below this value, the perturbation induced can be distributed all over the nuclear system during collision time. In other words, thermalization can be achieved but above this value it is impossible and thus the nucleons ejected remain very energetic.

Fourth, it has long been known that two nuclei can fuse only if the total energy transformed into excitation energy of the compound nucleus is not too high. Above an average value of 8 MeV per nucleon, the nucleons become unbound and the nucleus is then beyond its boiling point. Such a situation is reached in a collision of nuclei with similar masses when the bombarding energy in the laboratory reference frame is ~ 30 or 35 MeV AMU^{-1} but never in the few experiments performed above 100 MeV AMU^{-1} .

First results from GANIL

The long-term goals of the research programme at GANIL proceed from the above arguments. The energy available allows us to explore the transition region where the nuclei evolve from

a coherent state to a collection of individual nucleons. One can formulate the regime in terms of a thermodynamic phase transition. The nuclear system formed in low-energy collisions can be described by collective variables; it exhibits a high degree of coherence and is not unlike a liquid. The concepts of viscosity, temperature, friction and the shape parameters seem to be useful. In high-energy collisions, the system behaves like an assembly of loosely interacting individual nucleons, rather similar to a gas.

The GANIL energies encompass the transition region. How does such a phase transition proceed and what phenomena occur when the nucleus loses its coherence? To observe and analyse these features is the primary experimental objective of the accelerator. More fundamentally, perhaps, these observations should allow physicists to address the following question: out of the very large number of degrees of freedom of the system formed by the excited assembly of nucleons, can the few relevant parameters that describe its structure and evolution be identified? This question defines a basic problem in statistical physics. The excited piece of nuclear matter formed in the collision has very distinctive features: it consists of a finite number of fermions, carries a high internal energy and is governed by the laws of quantum mechanics. Present-day statistical mechanics have not answered to this problem. It cannot provide parameters to derive the relevant variables nor laws to describe the evolution of such a system. These answers must come from nuclear physics itself.

Yet the experimental information obtained from these nuclear collisions seems hopelessly complex. A large variety of nuclear fragments with a wide energy spectrum are emitted at all angles and an accurate description of the actual reaction mechanism must be obtained from this mass of information. In heavy-ion physics, the additional wide variety of target, projectile and energy combinations adds both to the amount of information and to the difficulties of its interpretation.

The search for a thorough description of nuclear matter at the limits of its coherence, which, as stated above, is the goal of GANIL physics, could then start from hundreds of arbitrarily chosen points of view. One could say: let us investigate whether fast oxygen fragments are emitted forwards in coincidence with isotopically emitted α -particles, when argon and uranium nuclei collide. One would certainly observe such events with a probabil-

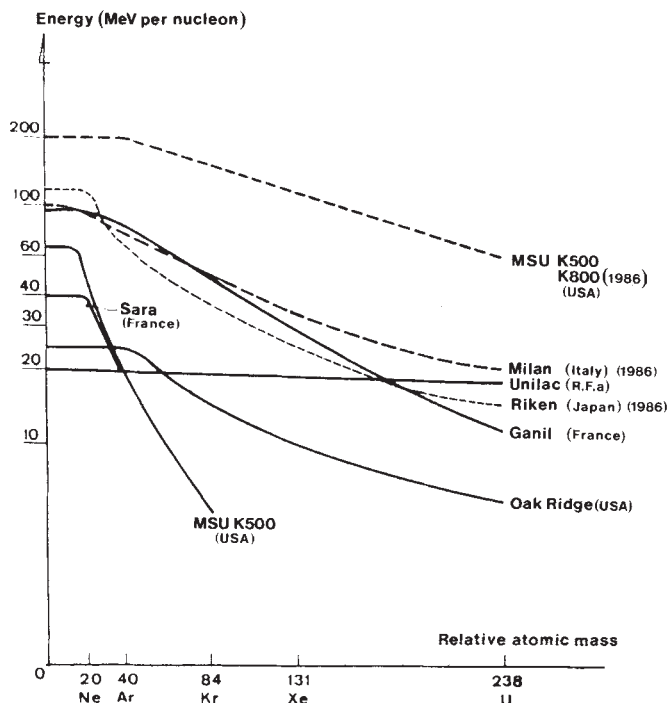


Fig. 2 Maximum energy (in MeV per nucleon) of accelerated ions from several accelerators, as a function of the atomic mass of the ion. Solid lines refer to operating facilities and dashed lines to projects.

ity that could be measured, then move to another event. However, little insight would emerge from such a random approach.

At GANIL, physicists started from known facts. As stated above, they had reached a detailed understanding of the dissipative phenomena that occur in lower energy collisions. Similarly, the well-documented fragmentation process observed at high energy could be used as a reference. These initial points of reference have been used extensively since the first experiments of January 1983.

In dissipative interactions, the two colliding nuclei are linked for a time, rotating around their centre of mass and progressively equilibrating the various parameters that describe their respective structure, such as the ratio of neutrons to protons (N/Z), the energy and the angular momentum. Of these parameters, the one that equilibrates fastest is the N/Z ratio. This means, for instance, that a ^{28}Si projectile (14 p, 14 n) interacting with a heavy target such as ^{208}Pb , which has a large neutron excess (82 p, 126 n), will emerge as a neutron-enriched fragment. Such an equilibration occurs in $\sim 2 \times 10^{-22}$ s. It seemed of interest to see whether such a fast equilibration process would still occur at higher incident energies, that is, for shorter interaction times.

An unambiguous answer was achieved with incident beams of ~ 40 MeV per nucleon. Although the interaction times are long enough to accommodate the fast N/Z equilibration process, the average value of the N/Z parameter of the emitted fragment was found not to be influenced by the target. Rather, it closely reflects the value of the projectile, hence pointing to a fragmentation process. The forward-emitted nucleus results from the breaking of the projectile in the field of the target. No binuclear state is formed that would allow for some equilibration of the parameters. It took only two weeks to obtain the expected, although reassuring, information that the GANIL energies were high enough to observe the transition from low energy to high energy processes; it took a few months to confirm it with other projectile-target systems. Since then, however, a large amount of beam time has been necessary to qualify the observation that, at energies as low as 40 MeV per nucleon, collisions result in fragmentation.

Several well-established properties of the forward-emitted fragments are incompatible with a pure projectile-fragmentation

mechanism. First, it was observed that the isotope distribution of the fragment (that is, the variation of the yield of isotopes of a given element Z with their mass A), depends somewhat on the nature of the target. More precisely, the more neutron rich the target is, the more probable is the emission of a neutron-rich fragment. Hence, the so-called fragmentation of the projectile includes some exchanges between the two colliding nuclei, a property obviously at variance with a pure fragmentation process. Second, some of the forward-emitted fragments actually gained one or two neutrons or protons as compared with the incident projectile, which again is incompatible with pure fragmentation. Finally, the energy characteristics of these fragments differ from those observed at higher energy. Instead of being centred around the velocity of the projectile, as expected and observed for fragmentation products, the velocity spectrum extends to lower values, giving evidence for the importance of relaxation processes during the interaction.

This accumulation of facts for and against a model of a reaction discussed here must be seen as an example of the approach pursued at GANIL during the first one or two years. Similar studies have been performed on the fate of central collisions. The ability of the two nuclei to fuse and the possibility that the resulting nucleus absorbs all the momentum carried by the projectile have been studied for different systems and for various energies.

This first stage of research is basically complete, at least for projectiles no heavier than krypton. The main facts are collected and published, but, at this stage, the conclusions remain ambiguous and difficult to formulate. This should not be surprising. Again, the results have been interpreted within the framework of known reaction mechanisms. Physicists have been looking for footprints they could identify and so they ascribe the results to the contributions of several mechanisms. This situation will not last: at the limits of nuclear coherence, when the internal motions inside nuclei and the relative motion of the two colliding nuclei carry similar velocities, new processes, as yet unknown, will provide a simpler description. So far we approximate them through conceptually understood mechanisms described for other situations. But the procedure that helps us describe the apparently complex properties of the system should not be mistaken for the as yet undefined new set of original concepts, effective variables and simplifying models.

Future research

We have now entered a new phase of development along the lines discussed above. Both experimental and theoretical physicists (perhaps not as readily distinguishable in heavy-ion physics as in other fields of nuclear physics) have concentrated on examining systematically the relevance of the variables commonly used in the light of the first results. For example, can we define a temperature and find unambiguous ways to measure it in the systems under study? This new phase should bring us closer to selecting the relevant parameters.

Simultaneously, the need to move from inclusive measurements to more exclusive ones results from the gross features of reactions already observed. For instance, when one observes a so-called fragment of an argon projectile after collision with, say, a nickel nucleus, which other fragments or particles emitted during that process have gone unobserved; at which angles were they emitted; and how much of the initial energy did they carry?

These questions call for large multidetectors with an ability to detect and measure fragments in all directions. For each single collision, several hundreds of parameters must be read to collect all the information gathered by all the detectors. It would have been unrealistic to begin with such capabilities: how could intelligent use of 700-dimensional information be made? Now that the basic features of the reactions have been observed, such second-generation studies become not only legitimate but necessary. These new experiments have already started at GANIL, where >200 parameters can be read. For example, two-proton interferometry is used to study the size of the emitting hot zone of the so-called participant nucleons described earlier.

This method is very similar to the two-photon interferometry used to measure the size of stars.

We are ready to develop detectors and data-acquisition that can measure up to 700 parameters. I do not believe that this implies a blind jump into experimental megalomania. This programme is extrapolated from systems already operational and derives naturally from results obtained by physicists already involved in understanding the dynamics of the collisions.

Here, I have not given a full review of all the results obtained and all the ideas advanced to understand them, but have attempted, by the use of examples, to outline the means by which original properties of nuclear matter in the transition energy region opened by GANIL are being investigated. Indeed, this is the major challenge, yet the potential of high-intensity medium-energy heavy-ion beams for nuclear physics is, in my view, already well established by some of the results obtained. The use of projectile fragmentation to observe new neutron-rich

isotopes through a triple-focusing magnetic analysis system allows us to extend the limits of known nuclei by one or two isotopes: ^{23}N , ^{29}Ne and ^{30}Ne were seen in the first attempt. The high excitation energy that can be transferred to nuclei has allowed the observation of high-lying excited states of simple configuration, probably multi-phonon excitation, of up to ~ 70 MeV. The cooperative creation of pions below the nucleon-nucleon threshold has been observed at the lowest energy ever.

Those are only some examples of the results obtained. In the coming years, several new machines at Catania, East Lansing (Michigan), Tokyo, Lanzhou and elsewhere will achieve the performance of GANIL. This interest reflects the novelty and scientific importance of a wide field now opened. One can anticipate that major developments in the understanding of the properties of nuclear matter will result from such a large-scale effort. The challenge for the GANIL physicists will be to play a leading role in the emergence of the new ideas.

ARTICLES

A quantum potential approach to the Wheeler delayed-choice experiment

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We apply the quantum potential approach to a 'delayed-choice' experiment considered by Wheeler and show that there is no need to conclude that the past has had no existence except insofar as it is recorded in the present.

WHEELER¹ has given a detailed description of several possible types of 'delayed-choice' experiment designed to show that, according to the quantum theory, the choice to measure one or another complementary variable at a particular time can apparently affect the course of events for considerable periods of time before such a decision is made. Wheeler interprets this using Bohr's principle of complementarity and his emphasis on the phenomena and experimental arrangement as forming an indivisible whole. He concludes that "no phenomenon is a phenomenon until it is an observed phenomenon", so that the "Universe does not 'exist out there' independently of all acts of observation. It is in some strange sense a participatory Universe. The present choice of the mode of observation... should influence what we say about the past..., the past is undefined and undefinable without the observation."

We discuss here the same question raised by Wheeler using a different approach that is completely consistent with quantum mechanics, namely the quantum potential approach^{2,3}. (This question has also been treated by Bell⁴ who came to a similar conclusion emphasizing different aspects of the problem.) Our approach agrees with Wheeler in concluding that this is a participatory Universe but differs in that the whole process, past and present, is treated as uniquely determined in itself, and not merely by what we are led to say about it through our observations. That is to say, there is a definable and defined overall process that includes both the observer-participator and the rest of the Universe in one undivided whole. Thus, we concur that the behaviour of the Universe is not independent of acts of observation, but also add that the past has had an existence independent of these acts.

Delayed-choice experiment

To bring out the difference between Wheeler's approach and our own through the quantum potential model, we will first

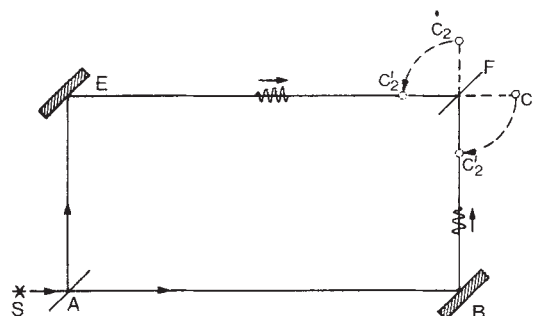


Fig. 1 Schematic view of the delayed-choice experiment for photons passing through a half-silvered mirror at A. Counters C_1 and C_2 can be moved into positions C_1' and C_2' .

re-examine one specific experiment described by Wheeler (the generalization to other more complex experiments is straightforward).

Two photodetectors are mounted so that they can be moved easily and quickly either to positions C_1 and C_2 or to the positions C_1' and C_2' (Fig. 1). Suppose a single quantum of electromagnetic energy passes through a half-silvered mirror at A. Then, with the detectors in positions C_1 and C_2 , the relative counting rates will determine the phase difference between the two routes. With the counters in positions C_1' and C_2' , one can infer which route the photon takes. Thus, we can choose whether to perform either a two- or a single-beam interference experiment by the choice in the positions of the two detectors. Furthermore we can make this choice some time after the original photon has entered the apparatus but before it reaches F, thus giving rise to a delayed-choice effect. It is as if we could determine by our choice whether the photon has travelled along both routes or only one route.

It should be emphasized that Bohr has already pointed out that, in his view, the notion of the route that the photon or

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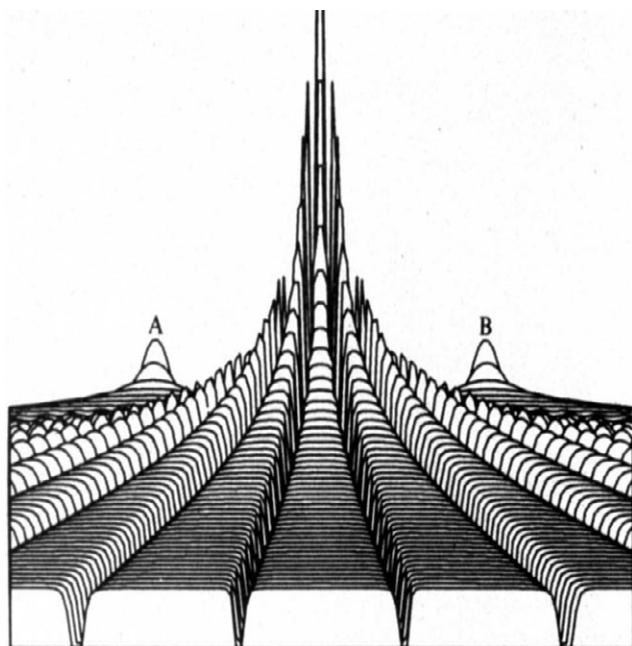


Fig. 2 Quantum potential for the two-slit experiment. The two slits are at A and B while the interference fringes appear in the foreground.

particle travels along has no meaning. Indeed, he⁵ cites this very experiment, and in commenting on it, states, "it is just arguments of this kind which recall the impossibility of subdividing quantum phenomena and reveal the ambiguity in ascribing customary physical attributes to atomic objects". He further states that "all unambiguous use of space-time concepts in the description of atomic phenomena is confined to the recording of observations which refer to marks on a photographic plate or to similar practically irreversible amplification effects like the building of a water drop around an ion in a cloud-chamber".

The very idea of a 'micro-reality' (such as photons, electrons) that exists in some sense independently of the experimental context is thus denied. To be consistent with Bohr's point of view, the discussion should be solely in terms of what is observable and no attempt should be made to discuss paths of particles that would provide an explanation or account of how the experimental results obtained at successive times are connected.

Heisenberg⁶ has a view similar to that of Bohr in many respects but different in that he argues that it is meaningful to talk about an orbit after an observation has taken place. In fact he writes "... the 'orbit' comes into being only when we observe it". For example, if an electron is observed at A and then later at B, one could say that the orbit went from A to B but the subsequent motion of the electron beyond B would be unpredictable. A further measurement at C might show that it actually was, but then the movement beyond C would be unpredictable. Thus, the concept of an orbit has a meaning only in the past of the act of observation.

Note, however, that there is no way to verify that the inferred trajectories were the actual orbits by additional experiments. Thus, it would be quite possible that the particle did not follow a straight line between A and B or, indeed, that there was no particle at all in the intermediary space and time. It would be equally consistent to assume that the particle appears only in the act in which it is made manifest. Thus, this discussion of the paths of particles has only the function of giving an explanation of how the phenomena are connected and is not capable of being verified experimentally.

Wheeler, of course, agrees basically with Bohr's point of view, but he has used Heisenberg's way of discussing this situation to show that the assumption that the past did exist independently of present observation leads to absurdities. This he regards as a support for his notion "that no phenomenon is a phenomenon

until it is an observed phenomenon". In doing this, Wheeler does not use specific particle trajectories, but instead uses wave-packets to connect the present to the past. When the particle does not show interference effects, this packet corresponds approximately to a somewhat indistinctly determined trajectory, but when the experimental arrangement allows interference, the connection between the present and the past is made by the two packets acting together.

The approach of Bohr and Wheeler is possible in the sense that it is consistent. We feel, however, that the quantum potential approach, which we will outline below, treats the whole question in a more intelligible way. Here it is consistent to say that the past existed in a unique way that does not depend on what happens in the present. Nevertheless, some of the key points raised by Wheeler are retained but are comprehended differently.

Quantum potential approach

A consistent treatment of the electromagnetic field using the quantum potential approach is possible, and the outline of such a treatment has indeed already been elucidated^{2,7}. However, the details are rather complicated and would perhaps obscure the main point. Therefore, we confine our attention in this paper to the interference of particles such as electrons and neutrons, for which we can apply the quantum potential approach in a straightforward way⁸. Indeed, delayed-choice experiments of the type suggested by Wheeler, but using neutrons, have actually been proposed by several authors^{9,10}.

We begin by first writing the wavefunction in standard polar form

$$\psi(\mathbf{r}, t) = R(\mathbf{r}, t) \exp \left[\frac{iS(\mathbf{r}, t)}{\hbar} \right] \quad (1)$$

From Schrödinger's equation it follows, writing $P = R^2$, that

$$\frac{\partial P}{\partial t} + \nabla \cdot \left(\frac{P \nabla S}{m} \right) = 0$$

and

$$\frac{\partial S}{\partial t} + \frac{(\nabla S)^2}{m} + V + Q = 0 \quad (2)$$

where Q is the quantum potential given by $Q = -(\hbar^2/2m)(\nabla^2 R/R)$ and the velocity is assumed to be given by $V = \nabla S/m$.

To interpret these two equations, we regard, say, a neutron as consisting both of a particle, which has a definite position and a definite momentum (and therefore a definite trajectory), and a field which is inseparable from the particle. The field is just the wavefunction as expressed through the two real fields R and S . It satisfies the Schrödinger equation which determines the time behaviour from the initial conditions.

Before proceeding further, we stress again that the particle and the field are indissolubly linked and we make no assumptions as to why or how this arises. Here we are simply attributing to the field those properties that are necessary to account for the quantum phenomena. To explain the origins of this field will require us to go deeper and make new physical assumptions of the type used by Bohm and Vigier¹¹ or by Cufaro Petroni and Vigier¹² or, indeed, some other appropriate ideas. It is not necessary to address such questions here as they are not relevant to the principles we wish to discuss. As a result, we use concepts such as 'wave-packet' that are applicable in the one-body case, but cannot be generalized in such a simple way to the many-body system which would require a field in the multi-dimensional configuration space. Therefore, such concepts are simply a convenient way of dealing with the phenomena and should not be taken as a definitive description of the nature of reality at this level.

Returning now to the main discussion, it is evident that equation (2) is similar to the Hamilton-Jacobi equation but it differs in that it has the additional quantum potential term Q , which leads to the suggestion that the particle follows an orbit different from the classical one. This difference is the result of

the quantum potential and is one of the major factors accounting for the quantum properties of matter.

To account for the rest of the quantum properties of matter, we must first consider the single valuedness of the wavefunction that leads to a kind of modified Bohr-Sommerfeld quantum rule

$$\oint \nabla S \cdot dx = nh \quad (3)$$

(The difference from the Bohr-Sommerfeld quantum rule is that, in the former, S is obtained from the classical Hamilton-Jacobi equation, whereas here it is obtained from the quantum Hamilton-Jacobi equation (2).)

Finally, we have to interpret equation (1), which is evidently a conservation equation. We can do this consistently by assuming a statistical ensemble of similar systems all having the same wavefunction ψ . We further postulate that P is the probability distribution in this ensemble. This postulate is consistent in the sense that if it holds at the time $t=0$ the conservation equation will guarantee that it will hold at all times.

Wigner¹³ has objected to this approach by arguing that Q depends on R and that R refers only to the ensemble of systems. He concludes that it is unreasonable to have a theory in which the individual depends on the ensemble. Our answer to this criticism is that we do not primarily regard R as referring to the ensemble but rather as part of a field that belongs to the individual system as much as the particle does. The major effect of the field is to produce the force derivable from the quantum potential. But we have shown that the field can also describe the probability in an ensemble of systems that have the same field; that is, that have the same wavefunction except for a constant factor. This requires, of course, a further special assumption on the statistical ensembles. But the consistency of this assumption depends on the properties of the individual systems that make up the ensemble and, therefore, the individual does not depend on the ensemble but the ensemble depends on the individual in the way that is customary, for example, in classical statistical mechanics. The only new feature is that both quantum potential and probability are obtained from the same field R .

As an example, we give here the two-slit interference experiment¹⁴. We assume as an initial condition an ensemble of particles each having a plane wave that passes through a pair of slits. After passing through the slits, the two waves interfere and produce a very complex quantum potential shown in Fig. 2. At some distance from the slits, the form of this potential is that of a set of plateaus separated by a set of deep valleys. Figure 3 shows the paths of the particles in the ensemble. Clearly the net effect of the potential is to bunch the trajectories so as to produce the pattern of the well-known interference fringes in the statistical ensemble.

To understand this result, it is crucial to note that the quantum potential is not changed when the wavefunction is multiplied by an arbitrary constant, that is, that the quantum potential is determined only by the form of the wavefunction and not by its magnitude. This means that the effects of environmental features such as the slits can be felt by the particles at long distances. This is not in itself a form of non-locality because the effect is propagated locally via the wavefunction to which Schrödinger's equation applies. However, it represents the possibility that the behaviour of the particle cannot be separated from the whole context of the environment which is involved, for example, through a determination of the experimental conditions. We also add here that through such a dependence on the environment, the particle does not necessarily travel in a straight line at constant speed in what appears to be empty space. Rather, as shown in Fig. 3, it may have a complicated and irregular motion.

Let us now proceed to use this approach to account for Wheeler's¹ experiment. A detailed treatment using the quantum potential would be more complex than that required for the two-slit experiment described above, but the basic principles involved are the same. Therefore, we can use the approach to account in a general way for the experiment shown in Fig. 1.

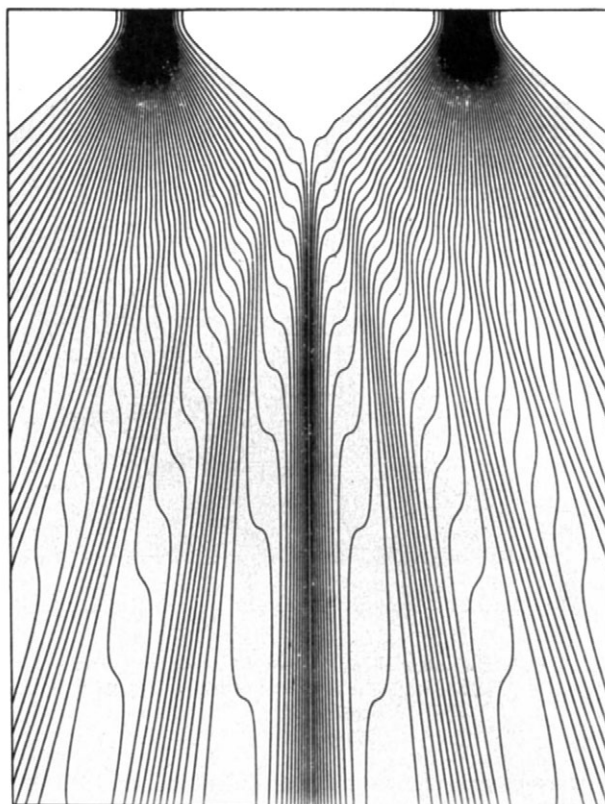


Fig. 3 Particle trajectories for particle passing through the two slits at the top and forming interference fringes at the bottom.

Each individual member of the ensemble goes one way or the other. That is, one of the wave-packets contains the particle and the other does not. As explained in our discussion of the measurement², if there is a particle detector for each packet, then the one actually containing the particle will be the one that registers. If, however, we allow interference to occur before we detect the particles, there will be a region of complex fluctuating quantum potential where the wave-packets overlap. When the particles enter this region, the trajectories will no longer be straight lines but rather will bunch into interference fringes in a way similar to what happens in the two-slit experiment. If the particles are detected, then the ensemble will show the interference pattern¹⁵.

In no case is the behaviour of the system in the past modified. Each individual system will develop two wave-packets after passing through the half-reflecting mirror A (see Fig. 1). In each case the particle will go one way or the other, while the wave-packet is both transmitted and reflected. Indeed, which wave-packet the particle enters at A is determined by its (unknown) initial position in the incoming wave-packet (as can be shown by considering the scattering of a wave-packet from a square potential barrier¹⁶). No matter what is done later on in the experiment, for example, moving the pair of detectors from one position to another, this is not altered.

Until the two wave-packets travelling the two routes to F overlap, each particle follows a trajectory determined by a quantum potential independent of the existence of the alternative route. Detection at this stage by C_1' and C_2' simply gives information concerning which route the particle took. When the wave-packets overlap, the resulting quantum potential is determined by the physical conditions along both routes. In this region the trajectory of the particle is modified in such a way that the ensemble of trajectories produces an 'interference pattern' even though the particle has only travelled along one path. However, what we do with the detectors can affect the quantum potential that each particle experiences between the mirror and

the detector and in this way we obtain either a simple distribution of particles between alternative wave-packets or an interference pattern.

Conclusion

Here, we have shown that, using the quantum potential approach, it is possible to give a simple and intelligible account of a typical delayed-choice experiment. It is not necessary to suppose that the behaviour that we ascribe to a system in the past will depend in any way at all on what is done to it later. As we stated earlier, we feel this is more satisfactory than the approaches either of Heisenberg, who says that the orbit comes into being only when we observe it, or of Wheeler, who implies that the past has had no existence except in as far as it is recorded in the present.

Of course, to achieve this result we had to assume the particle trajectories that are not observable in detail, at least in any domain in which quantum mechanics is valid¹¹. In this respect, our approach is similar to that of Heisenberg, who makes statements about the past that are also unverifiable by observation to give an account of how certain phenomena at different times may be related. However, by bringing in the quantum potential, we are able to understand the characteristic results of the quantum theory; for example, interference¹⁴, barrier penetration¹⁶, the Einstein-Podolsky-Rosen situation¹⁷, the Schrödinger cat paradox and the measurement problem².

In this regard, our trajectories have essentially the same purpose as the trajectories of Heisenberg (although we think that our trajectories achieve much more). We do, however, find it difficult to understand why many authors reject the quantum potential approach on the grounds that it provides no new experimental consequences beyond those of the usual theory, while they are ready to engage in discussions of what may happen between measurements, discussions that equally have no further experimental consequences. Rather we agree with Wheeler who says "We search here not for new experiments or

new predictions but for new insights". In this connection, we emphasize also that we are not proposing the quantum potential as a definitive explanation of the nature of reality. We do appreciate that the quantum potential is an arbitrary assumption, but think that the use of this assumption helps to understand the meaning of the quantum theory in an intuitively clear way. Many questions that are unclear in the usual interpretation (for example, the Einstein-Podolsky-Rosen experiment, the experiment discussed in this paper) became obvious in meaning when put in terms of the quantum potential.

Moreover, this interpretation gives results entirely compatible with Bohr's views on wholeness and complementarity. We regard our approach as complementing Bohr's instead of opposing it. To support this, we point out that our approach is generally more consistent with the spirit of the interpretations of Bohr and Wheeler than it is with the one adopted by Heisenberg. In other words, if one does want to use some sort of trajectory to explain the meaning of quantum mechanics, then the quantum potential is necessary if this is to be consistent and fully compatible with the experimental meaning of Bohr's interpretation.

Finally, as stated in the introduction, we agree with Wheeler that this is a participatory Universe. However, we suggest that this comes about because the observer-participator in, for example, determining the environmental context needed to observe a particle, creates a situation in which the fundamental mode of behaviour of the particle is strongly affected by this context (for example, the slit system can bring about a very irregular trajectory even when the particle is far away from it). Thus, the Universe cannot be analysed into elements that are essentially independent when they are far apart. In this way, the participation of the observer in the rest of the Universe (and of the rest of the Universe in the observer) has a far more profound significance than it has in classical physics. Through the quantum potential a certain insight into this significance has been made possible.

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Role of lithospheric strength heterogeneities in the tectonics of Tibet and neighbouring regions

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Unlike rigid oceanic plates, continents show diffuse deformation, for example the Tibetan plateau that has accommodated much of the convergence of India with Asia. We interpret the Tertiary deformation of Asia as the three-dimensional strain of a non-linear, viscous continental lithosphere. Lateral strength contrasts modify this deformation; stress concentration around the relatively strong Tarim basin gives rise to local crustal thickening in the Tien Shan to the north and to strike slip in the Altyn Tagh along its southern margin.

THE region accommodating the convergence between India and the Asian landmass is one of the clearest examples of non-plate-like behaviour of the continental lithosphere¹. This deformation has been explained in several different ways. One interpretation

involves the thrusting of India under the entire Tibetan plateau²⁻⁴ but, although there is some present-day underthrusting of Indian crust underneath Tibet, recent seismic reflection^{5,6} and palaeomagnetic^{7,8} studies suggest that most of

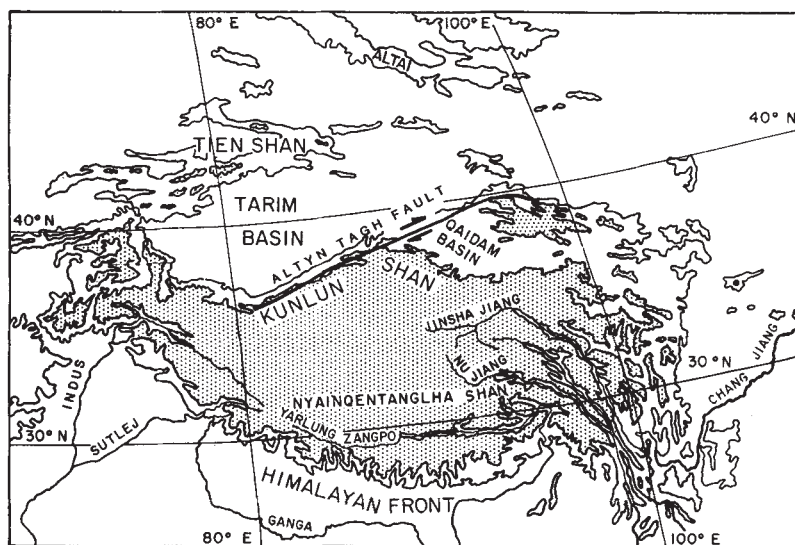


Fig. 1 Locations of principal features referred to in this study. The 2,000- and 4,000-m contours are shown, with land above 4,000 m stippled; the position of the Altyn Tagh fault is taken from the map of Tapponnier and Molnar⁹, to which reference should be made for positions for other major faults in the region.

the post-Eocene convergence between India and Asia has been taken up by shortening in Asia.

Continuum models for this intraplate deformation fall into two groups. The first emphasizes the role of strike-slip faulting in the present-day tectonics of Asia and interprets the deformation in terms of the predictions of plane-strain slip-line calculations⁹, or by analogy with unscaled plane-strain plasticine models¹⁰. The second group represents the continental lithosphere by a thin non-linear viscous shell that can be analysed quantitatively as to its predictions about crustal thickness, strain and strain-rate fields and other parameters of finite deformation^{11,12}. This approach recognizes the three-dimensional nature of the Tertiary deformation in Asia and accounts for the relatively greater importance of lateral motions at the present day in terms of the buoyancy forces generated by the elevation contrasts produced during the thickening of the Tibetan crust^{9,12,13}. What these calculations have not contained so far is an analysis of the effect of a large-scale heterogeneity that is represented, for example, by the relatively aseismic and apparently undeforming Tarim basin¹⁴ (Figs 1, 6). Here we pursue the implications of the thin sheet approach to continental tectonics^{11-13,15,16} by assessing the influence of heterogeneities in lithospheric strength on the tectonics of Asia.

Mathematical model

The thin-sheet model has been described in detail elsewhere¹²; the lithosphere is represented by a thin layer of non-newtonian viscous fluid; for mathematical tractability we assume that horizontal gradients of crustal thickness and topography are small and use a depth-averaged constitutive relation of the form

$$\bar{\tau}_{ij} = 2\eta\dot{\epsilon}_{ij} \quad (1)$$

where $\bar{\tau}_{ij}$ is the depth-averaged deviatoric stress component, $\dot{\epsilon}_{ij}$ is the corresponding strain rate and η is the depth-averaged effective viscosity

$$\eta = \frac{B}{2} \dot{E}^{(1/n-1)} \quad (2)$$

where $\dot{E}$ is the second invariant of the strain-rate tensor and B is a depth-averaged strength coefficient. In the experiments described here, $n > 1$, so the effective viscosity is a function of position in the solution region.

The horizontal velocity components are assumed to be independent of depth in the layer and the solution is constrained by boundary conditions on the stress or velocity as shown in Fig. 2. The force balance equations for creeping flow are solved by finite element techniques.

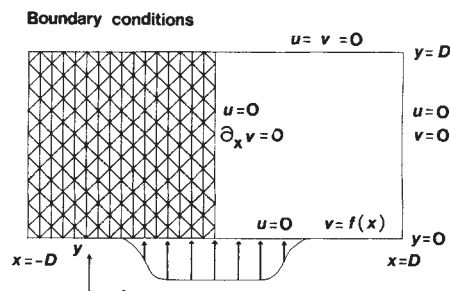


Fig. 2 Initial geometry, coordinates and boundary conditions for the numerical experiments. The x - y plane is horizontal, the line $x = 0$ is an axis of symmetry and the region is divided into triangular elements as shown in the left. Velocities are zero on the boundary, except on the part of it that initially coincides with the x -axis, where the region $-D/2 < x < D/2$ moves in the y -direction, representing the edge of an indenter. The motion of this boundary deforms the sheet (see Fig. 3). The velocity function that describes the indenter is: $v(x) = 0$ for $D/2 < |x| < D$, $v(x) = u_0$ for $0 < |x| < D/4$ and $v(x) = u_0 \cos^2(2\pi(x/D - 1/4))$ for $D/4 < |x| < D/2$. For convenience in the text, the positive y -direction is referred to as north, the positive x -direction as east, etc.

The rate of thickening or thinning of the crust is determined locally by the horizontal divergence of the calculated velocity field

$$\frac{1}{S} \frac{\partial S}{\partial t} = -\nabla \cdot \mathbf{u} \quad (3)$$

where S is the thickness of the crust and $\mathbf{u}$ is the velocity. Under the influence of gravity the thickened crust tends to spread horizontally, introducing a force proportional to the gradient of the square of crustal thickness into the dimensionless stress balance. The constant of proportionality is called the Argand number (Ar) by England and McKenzie¹¹ and is a measure of the magnitude of this gravitational spreading force relative to the viscous resisting forces.

The constant B (equation (2)) contains the depth averaged temperature dependence of the effective viscosity (see appendix of ref. 11) and hence is dependent on the geotherm, the depth to the Moho and lithological variations. Previous calculations¹¹⁻¹³ have assumed for simplicity that B is a constant. However, it has been suggested^{14,16,17} that regions such as the Tarim basin are stronger and more resistant to deformation than the surrounding continental lithosphere. In the experiments described here, we investigate the consequences of including a small region of anomalously high strength in an otherwise

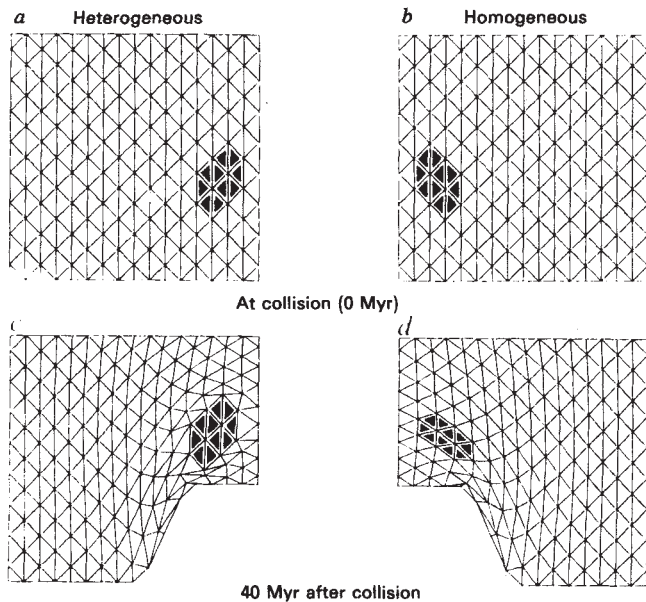


Fig. 3 *a, b*, Initial and *c, d*, final ($t = 40$ Myr) mesh configurations for the left and right halves, respectively, of the two symmetrical solutions for: *a* and *c*, the case where the effective viscosity coefficient (B , equation (2)) is increased by a factor of 10 in the shaded elements and *b* and *d*, the laterally homogeneous case, with the corresponding elements shaded for comparison with *a* and *c*. For both experiments $n = 3$ and $Ar = 3$.

laterally homogeneous layer; the constant B is increased by a factor of 10 in the shaded region of Fig. 3*a*. As a rough estimate of the effect of this modification, it follows from equations (1) and (2) that if the stress field is constant and the strain-rate field is uniaxial, then

$$\dot{\epsilon} \propto B^{-n} \quad (4)$$

For $n = 3$ the strain rates in the anomalously strong region would be only 10^{-3} of those in the surrounding regions. In fact, because of changes to the stress field (Fig. 4) and the triaxial nature of the strain-rate field, the contrast is in the range $0.5\text{--}1.0 \times 10^{-2}$.

Because of computation limitations, calculations were performed with an axis of symmetry along the perpendicular bisector of the indenter (Fig. 2). For the calculation shown in the left halves of Figs 3–6 there are two anomalously strong regions disposed symmetrically about the axis and having approximately the same positions relative to the indenter as do the Tarim and Qaidam basins to the arc of the Himalayan mountains (Fig. 1). We contrast these results with the calculation shown in the right halves of Figs 3–6 for the symmetrical laterally homogeneous case. By presenting in this format half of each solution, we mean to highlight the differences in tectonic style between western Tibet and Xinjiang province on the one hand and eastern Tibet and Gansu province on the other.

Results

We first describe the results of a calculation with $n = 3$ and $Ar = 3$, as these parameters give reasonable agreement with observation, but also note that effective values of $n \leq 10$ and $Ar \leq 10$ can give reasonable results (Fig. 6). Figure 3 shows the initial and final configuration ($t = 0$ and 40 Myr) of the finite element mesh for the two calculations. The vertices of each triangle are fixed to and carried along by the deforming medium. The strong region is barely deformed in Fig. 3*c* and has been translated and rotated practically as a rigid body; this behaviour should be compared with that of the corresponding region in the laterally homogeneous case (Fig. 3*b, d*).

The deviatoric stress fields in Fig. 4 show that the stresses in the strong region are amplified by the more rapid creep rates in the weaker material next to it (compare with ref. 18).

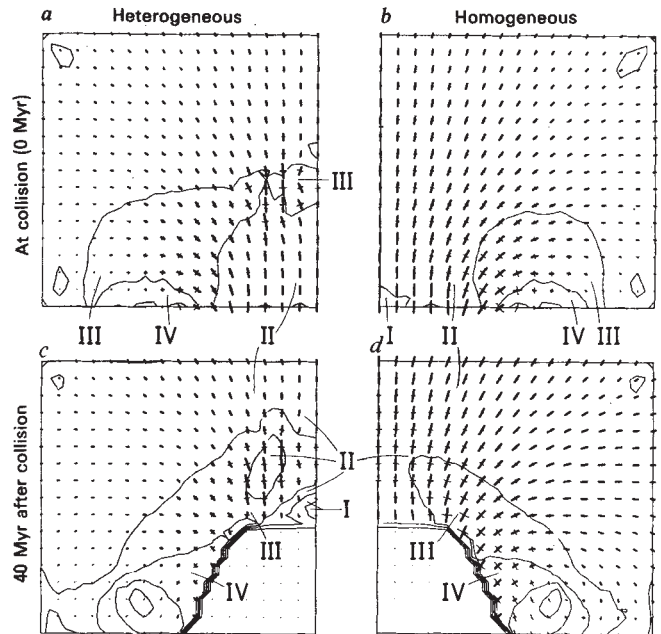


Fig. 4 Principal deviatoric stress axis orientation and magnitude for *a, b*, $t = 0$ and *c, d*, $t = 40$ Myr. The length of each arrow is proportional to the magnitude of the stress and the longest arrow represents a stress of *a*, 63.9 MPa, *b*, 43.5 MPa, *c*, 95.1 MPa and *d*, 44.2 MPa. Superimposed are contours of the parameter $\tan^{-1}(\dot{\epsilon}_2/\dot{\epsilon}_1)$, where $\dot{\epsilon}_1$ and $\dot{\epsilon}_2$ are principal horizontal strain rates. These contours divide the box into regions that show a consistent style of faulting (see text for II and III; I is a region of pure thrust faulting and IV is a region of normal plus strike-slip faulting).

At $t = 0$ the maximum stress in Fig. 4*a* is 47% greater than that of the corresponding laterally homogeneous case (Fig. 4*b*) and is found in the strong region rather than on the corner of the indenter. After 40 Myr (Fig. 4*c*), the maximum stress in the basin has increased by a further 49%. There is also a slight rotation of the principal stress axes near the viscosity heterogeneity.

Superimposed on the stresses of Fig. 4 are contours separating regions with a different style of surface faulting, assuming that the upper brittle crust deforms with the same strain rates as the underlying ductile layer. In region II, both strike-slip and thrust faulting must occur, although there is a greater rate of moment release from the thrust faulting component. In region III, the same two fault styles would be observed, but the rate of moment release from the strike-slip component is greater in this region.

For both calculations (with the strong region, Fig. 4*a, c*, and the homogeneous case, Fig. 4*b, d*) region III increases in lateral extent as the indenter moves further north, because as the crust is thickened the gravitational forces tend to resist further crustal thickening (thrust faulting) and an increasing percentage of the deformation is accommodated by strike-slip faulting^{11,13}. When the strong region is present, strike-slip deformation is predominant on its east and west margins throughout the deformation (Fig. 4*a, c*). At the centroid of the element next to the southern corner of the strength heterogeneity, the rate of moment release for thrust faulting is 3.7 times that for strike-slip faulting at $t = 0$, but at $t = 40$ Myr the strike-slip component is greater than that of the thrusting by 46%. For the same material point in the homogeneous calculation, thrust faulting is dominant throughout, although the ratio of thrust moment to strike-slip moment falls from 3.6 to 1.2 during the 40 Myr. The region next to the south-east margin of the basin, which at 40 Myr shows the maximum strain rates and strongest crustal thickness gradients (Fig. 6*a*), is thus found to be undergoing predominantly strike-slip deformation (Fig. 4*c*) during the last 10 Myr of the calculation. This region is in the same position relative to the basin as is the Altyn Tagh fault system relative to the Tarim basin (Fig. 1).

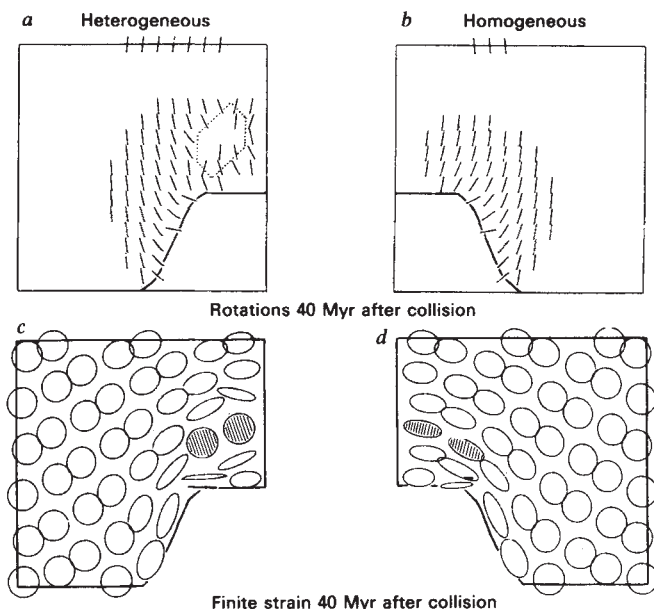


Fig. 5 *a, b*, Finite rotations (half of the time-integrated vorticity) at $t = 40$ Myr; *c, d*, finite deformation ellipses at $t = 40$ Myr; for *a, c*, the case with the strong region and *b, d*, the laterally homogeneous case. For *a* and *b* the angle between the small bar and the y -axis is the magnitude of the rotation; only rotations $> 5^\circ$ are shown. For *c* and *d* the ellipses show the finite strain, after 40 Myr, of initially circular horizontal material elements. The shaded ellipses in *c* lie in the basin and the corresponding ellipses are shaded in *d*.

Figure 5 shows the finite rotation and deformation in the two cases. Only rotations $> 5^\circ$ are shown so the strong region in Fig. 5*a* is defined clearly by the small magnitude ($\sim 5^\circ$) and opposite sign of its rigid-body rotation, compared with the anticlockwise rotation of $30\text{--}40^\circ$ of the material to its north-west and south-east. The rotations on the northwestern margin of the basin are significantly greater than they would be in the absence of the viscosity heterogeneity (Fig. 5*b*) and significant clockwise rotations ($\sim 20^\circ$) are also found on its north-east and south-west margins; otherwise the distribution and magnitude of rotation is much the same as in the homogeneous case.

Figure 5*c, d* is an alternative presentation of the data shown in Fig. 3*c, d*. Each ellipse shows the finite deformation undergone by a particular triangular element. The strain ellipses from the strong region (shaded) show ratios of major to minor semiaxes of 1.010 and 1.007 respectively, compared with the ratios for the corresponding ellipses in the homogeneous case of 2.39 and 2.35.

Figure 6*a, b* illustrates the elevations calculated for this model at 40 Myr after collision, compared with those observed in Asia at the present day. Because of the assumption of Airy isostasy in these calculations, contrasts in elevation are proportional to contrasts in crustal thickness. The contours of Fig. 6*a, b* show the most obvious effect of the viscosity anomaly; crustal thickening is inhibited within the strong region, whereas it is enhanced both to the north and south of the anomaly. Assuming isostatic balance, the topography would show a basin in the area corresponding to the anomalously strong region; after 40 Myr (Fig. 6*a*) the deepest part of the basin still has a 35-km-thick crust. To the south of the basin there is a steep topographical gradient where the crustal thickness increases by > 20 km as the indenter is approached and near the corner of the indenter the crustal thickness is more than doubled to 75 km. Immediately north of the indenter on the axis the crustal thickness is ~ 64 km, some 6 km thicker than for the laterally homogeneous calculation (Fig. 6*b*). To the north of the basin there is another local maximum of 55–60-km-thick crust. This local maximum bears the same geographical relation to the basin as does the Tien Shan mountain range to the Tarim basin (Fig. 1). In comparison,

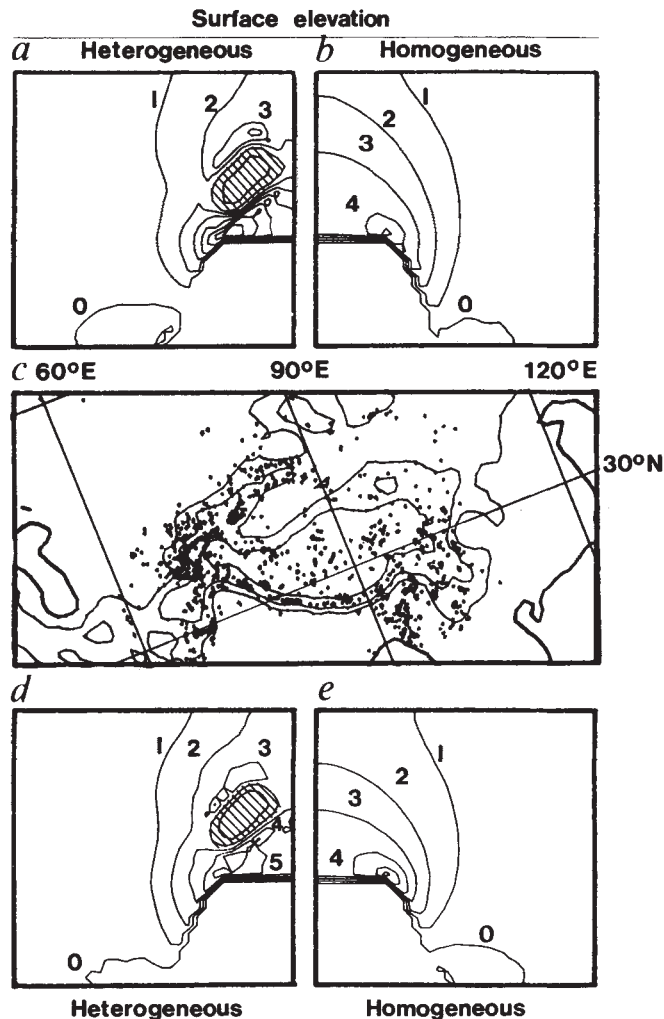


Fig. 6 Contours of elevation 40 Myr after collision for *a, d*, heterogeneous and *b, e*, homogeneous thin sheets, compared with *c*, contours of elevation in Asia. We assume an initial crustal thickness of 35 km, and changes in elevation ($e - e_0$) are related to changes in crustal thickness ($S - S_0$) by $(e - e_0) = (S - S_0) (1 - \rho_c / \rho_m)$. For $\rho_c = 2,800 \text{ kg m}^{-3}$ and $\rho_m = 3,330 \text{ kg m}^{-3}$ (ref. 22) difference in elevation of 1 km is equivalent to a difference in crustal thickness of 6.3 km in the model. In *a* and *b*, $n = 3$ and $Ar = 3$; the shaded region in *a* has a contrast in B of a factor of 10 relative to its surroundings. In *d* and *e*, $n = 10$ and $Ar = 10$ and the shaded region in *d* has B greater by a factor of 2 than its surroundings. Contours of topography in *c* are taken from Lee's²³ $1^\circ \times 1^\circ$ averages, smoothed with a gaussian filter of width 2° ; contouring intervals are 1 km. Dots indicate earthquakes in the interval 1964–1980, shallower than 100 km and reported to the International Seismological Centre by more than 25 stations. Note the relative quiescence in the seismicity of the Tarim basin.

the laterally homogeneous case (Fig. 6*b*) shows a monotonic decrease in crustal thickness northwards of the indenter.

A second important result of these calculations is illustrated in Fig. 6*d, e*, which shows the results of a calculation similar to that illustrated in Fig. 6*a, b* except that $n = 10$, $Ar = 10$ and the contrast in B for the strong region in the heterogeneous calculation is 2 (giving approximately the same contrast for uniaxial strain rates (equation (4)) as does a contrast of 10 when $n = 3$). With the minor difference that the peak crustal thickness near the corner of the indenter is decreased, the distribution of the topography in Fig. 6*c* resembles closely that of the topography in Fig. 6*a*. The resemblance emphasizes that the role of

heterogeneous inclusions in determining the scale lengths of continental deformation may be as important as that of the deformation mechanisms.

It would be difficult to distinguish, on the basis of the calculations shown in Fig. 6, between a model with a power-law exponent of 3, appropriate to a lithosphere governed by the deformation of olivine, and one with an effective power law exponent of 10, perhaps reflecting a significant contribution to the vertically averaged rheology from friction on faults.

Discussion and conclusions

The magnitude of the strength contrast required to produce the features described here is surprisingly small; for $n = 3$ a factor of 10 is more than adequate, and for larger values of n a smaller strength contrast would be sufficient. Assuming that the strongest part of the lithosphere is the uppermost mantle, we may use the power-law rheology given for olivine by Goetze¹⁹, and the expression of England²⁰ for the vertically averaged strength of a lithosphere governed by such a rheology, to estimate the drop in temperature at the Moho that is implied by increasing B (equation(2)) by a factor of 10. For $n = 3$ and Moho temperatures in the range 600–800 °C, the implied temperature decrease is ~100 °C, which is consistent with the suggestion of Molnar and Tapponnier¹⁴ that strength contrasts in the Asian lithosphere may be related to differences in geothermal regime in the region. However, we must caution against too literal

interpretation of these calculations in terms of a specific rheological model, in view of the many uncertainties involved in applying the results of laboratory measurements to the deformation of the continental lithosphere.

We have shown that the strain-rate fields and distribution of deformation in a thin viscous layer acted on by a rigid indenter are modified strongly by the presence of a strength heterogeneity in the layer. The most important features predicted by the calculations here are the preservation of a relatively low-lying region that is surrounded by thicker crust and higher topography, relatively small strain rates and total strain within this basin and a narrow zone of steep crustal thickness gradients and predominantly strike-slip deformation separating the basin from the plateau of much thicker crust to its south. It seems probable that if the symmetry requirement were dropped and the calculation were performed with a single heterogeneity corresponding to the Tarim basin, there would be a net eastward mass flux across the line that bisects the indenter and enhanced strike-slip motion at the southern edge of the high strength inclusion. The above features are all consistent with the interpretation of the Tarim basin as a relatively strong lithospheric block emplaced in an otherwise laterally homogeneous thin viscous layer; in this context the model explains the formation of the Tien Shan range and the Altyn Tagh strike-slip fault system. It seems likely that lithospheric strength heterogeneities (for example, the stable region of central Iran²¹) have similar roles in other collision orogenies.

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Abrogation of metastatic properties of tumour cells by *de novo* expression of H-2K antigens following H-2 gene transfection

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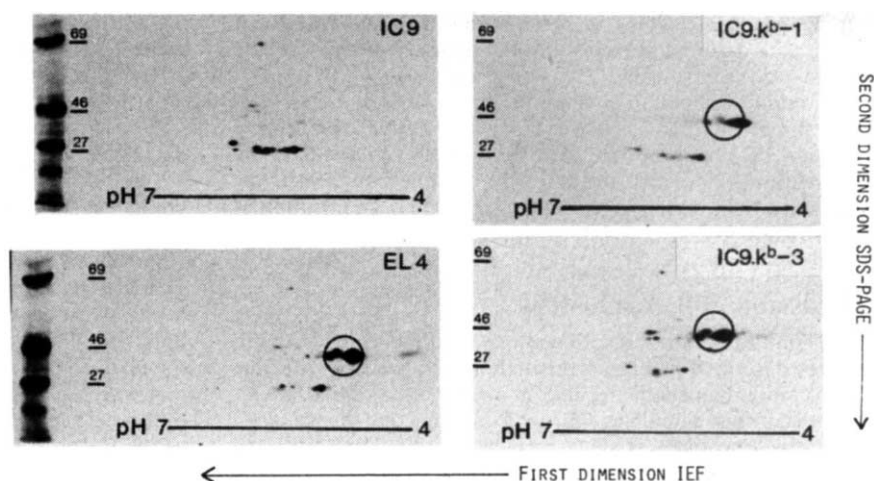
H-2 gene transfection was used to restore expression of H-2K antigens in metastatic and non-metastatic subclones of a murine fibrosarcoma that lack their major histocompatibility complex-encoded H-2K antigens. De novo expression of H-2K reduced tumorigenicity and abolished the formation of metastasis in syngeneic mice. Expression of H-2K may lead to effective recognition of the disseminating tumour cells by the host immune system.

THE major histocompatibility complex (MHC) of the mouse (H-2) encodes a group of cell-surface antigens that are involved in various pathways of the immune response. Thus, cytotoxic T lymphocytes (CTL) can recognize cells infected with virus or altered by neoplastic transformation only in association with an H-2 antigen (reviewed in ref. 1). It depends on the antigen as to which of the three known H-2 class I antigens, K, D or L, serves as a restriction element for CTL¹. Hence, it is conceivable that the control of tumour growth by the immune system would depend not only on the existence of tumour-associated cell-surface antigens, but also on the expression of MHC-encoded antigens on the tumour cells. Therefore, modulation of H-2

antigens of tumour cells may alter their growth properties because of alterations in the immune response of the host to the neoplastic cells.

Recent studies have demonstrated that the metastatic properties of certain mouse tumours are correlated with the relative expression of class I MHC antigens^{2–9}. A notable example is the methylcholanthrene-induced T10 sarcoma of (H-2^b × H-2^k) F₁ origin. Two distinct sets of T10 clones were isolated, differing not only in their metastatic potential but also in their expression of class I MHC antigens^{4,5}. According to their genotype, the tumour cells should express four different H-2 molecules, namely K^b, D^b, K^k and D^k. However, both sets of clones were

Fig. 1 Two-dimensional isoelectric focusing (IEF) gel of H-2K region associated antigens. $1-3 \times 10^6$ tumour cells (IC9, K^b -transfected IC9 and C57BL/6-derived EL4 lymphoma) were pulse labelled for 6 min at 37°C in the presence of $200 \mu\text{Ci } ^{35}\text{S}$ -methionine and lysed in ice-cold Tris buffer containing 1% Triton X-100. Cell lysates were preabsorbed with Protein A-Sepharose 4B for 2 h. H-2 proteins were immunoprecipitated using a mixture of monoclonal anti-H-2K^b antibodies K10-56, K9-136, K7-309, K9-178 (ref. 13) ($20\times$ concentrated culture supernatant) followed by Protein A-Sepharose for 5 h. Two-dimensional IEF gels were performed according to O'Farrell³⁰. Second-dimension gels were 10% acrylamide. Gels were processed with 2,5-diphenyloxazole dried, and exposed for 2 weeks using Kodak XAR-5 film. β_2 -Microglobulin, relative molecular mass 12,000, runs through the second-dimension gel and is therefore not visible on autoradiograms. Circles indicate positions of H-2K^b proteins. EL4 yields the same K^b spots as spleen cells from the C57BL/6 mice used for breeding of the C3H $\times$ C57BL/6 mice used in this study (not shown).



found to lack the K end-controlled K^b and K^k antigens. The non-metastatic clones express only the D^b molecule whereas the metastatic clones express both the D^b and D^k molecules (refs 6, 7; see also Table 1). Further studies suggested that the D^k molecule contributes to the metastatic potential of these clones⁸.

Here, we seek to determine whether expression of H-2 molecules encoded by the K end of the H-2 complex would influence metastatic spread and tumorigenicity of the T10 fibrosarcoma clones. To elucidate this problem we transfected clones of the T10 sarcoma with cloned H-2K genes and tested the malignant properties of the transfected tumour cells in syngeneic mice.

Restoration of H-2 expression

For transfection with H-2 genes a representative non-metastatic tumour clone (designated IC9) and a metastatic clone (IE7) were selected from the two sets of T10 fibrosarcoma clones. Cells were co-transfected with H-2K^b (ref. 10) or H-2K^k plasmid DNA¹¹ and plasmid pAG60 containing the neomycin resistant gene (*neo*^r) (ref. 12) and subsequently selected in medium containing Geneticin, which selects for cells that have productively integrated the *neo*^r gene. We identified colonies expressing transfected H-2 genes using specific monoclonal H-2 antibodies at a frequency of ~10–30%. After cloning of positive colonies,

Table 1 H-2 phenotype of transfected IE7 and IC9 tumour clones

Cell line	Transfected genes	Anti-D ^b	H-2 antibody (c.p.m. obtained)			H-2 phenotype
			Anti-K ^b	Anti-D ^k	Anti-K ^k	
IE transfected lines						
IE7	—	27,849	580	6,438	697	D ^b , D ^k
IE7.neo ^r -1	neo ^r	27,069	270	6,072	294	D ^b , D ^k
IE7.neo ^r -2	neo ^r	20,051	768	7,188	524	D ^b , D ^k
IE7.K ^k -1	neo ^r , K ^k	16,461	949	4,964	5,785	D ^b , D ^k , K ^k
IE7.K ^k -2	neo ^r , K ^k	21,176	683	5,382	7,413	D ^b , D ^k , K ^k
IE7.K ^k K ^b -1	neo ^r , K ^b , K ^k	16,160	13,664	5,539	6,992	D ^b , D ^k , K ^b , K ^k
IE7.K ^b -1	neo ^r , K ^b	10,447	9,350	3,433	851	D ^b , D ^k , K ^b
IE7.K ^b -2	neo ^r , K ^b	27,780	14,877	6,505	419	D ^b , D ^k , K ^b
IE7.K ^b -3	neo ^r , K ^b	17,350	12,631	4,817	913	D ^b , D ^k , K ^b
IC9 transfected lines						
IC9	—	28,259	453	397	507	D ^b
IC9.neo ^r -1	neo ^r	16,758	252	187	254	D ^b
IC9.K ^b -1	neo ^r , K ^b	10,629	15,944	848	653	D ^b , K ^b
IC9.K ^b -2	neo ^r , K ^b	18,118	14,496	414	478	D ^b , K ^b
IC9.K ^b -3	neo ^r , K ^b	21,806	28,420	719	718	D ^b , K ^b
IC9.K ^k -1	neo ^r , K ^k	17,023	514	745	4,282	D ^b , K ^k
IC9.K ^k -2	neo ^r , K ^k	19,316	471	516	10,305	D ^b , K ^k

Transfection was performed according to Wigler *et al.*²⁶ with some modifications. pBR322 plasmids containing the 10.5-kilobase (kb) *Eco*RI fragment of the H-2K^b and H-2K^k genes, respectively, were generously provided by Drs R. Flavell¹⁰ and B. Arnold¹¹. The H-2K^k gene (cos10.7) originally came from Dr M. Steinmetz. The plasmid pAG60 (ref. 12) containing *neo*^r was obtained from Dr B. Dobberstein. IC9 and IE7 cells were seeded at a density of $\sim 10^6$ cells per 10-cm Petri dish. Various mixtures of H-2 DNA (4 μg), *neo*^r (0.4 μg) and salmon-sperm DNA (20 μg), precipitated with calcium phosphate, were added. After 5 h at 37°C the medium was replaced by 5 ml 15% glycerol or a 41% solution of polyethylene glycol 4000 for 2 min. After overnight incubation with medium, the cells were collected with EDTA buffer and distributed into two COSTAR 3524 multiwell plates in selection medium containing 0.7 mg ml⁻¹ Geneticin (G418, Gibco). Growing colonies (1–10 per 10^6 input cells) were expanded, tested for H-2 expression by cytofluorography or radioimmunoassay and cloned by limiting dilution. (Without glycerol or polyethylene glycol almost no transfectants were obtained.) The table shows a representative cellular radioimmunoassay which was performed as described previously²⁷. Briefly, 10^6 cells were incubated in microtitre plates with specific anti-H-2 monoclonal antibody (anti-D^b, hybridoma B22-249, ref. 28; anti-K^b, K10-56, ref. 13; anti-D^k, 15-5-5S and anti-K^k, 16-3-22, ref. 13). After washing, ¹²⁵I-labelled Protein A was added and the amount of bound radioactivity determined. Values represent the mean of triplicate wells. The standard deviation ranged between 5 and 10% (not shown). Background with irrelevant antibodies was always $<1,000$ c.p.m. (not shown). The c.p.m. obtained with different H-2 antibodies cannot be compared because the affinity and amount of specific antibody in the hybridoma supernatant are probably different.

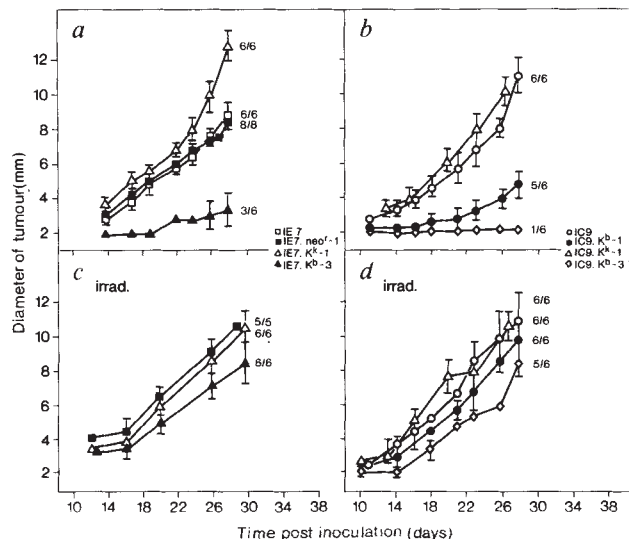


Fig. 2 Growth of parental and *H-2K*-transfected IE7 and IC9 cells. 10^4 *neo*^r and *H-2K*-transfected IE7 (*a, b*) or IC9 (*c, d*) were injected i.f.p. into intact (C3H.eB×C57BL/6)F₁ mice (*a, c*) and into sublethally irradiated (550 rad) F₁ mice (*b, d*). Tumour size was recorded using a caliper. The growth curves represent the mean tumour size of all mice inoculated in each experimental group $\pm$ s.e.m., including the diameter of legs of mice which rejected the inoculated cells. Mean diameter of legs of control non-inoculated mice is 2 mm. The numbers designated above each growth curve represent the proportion of mice that developed a tumour of the entire experimental group. *a, b*, □, IE7; ■, IE7.*neo*^r-1; △, IE7.*K*^b-1; ▲, IE7.*K*^b-3. *c, d*, ○, IC9; ●, IC9.*K*^b-1; △, IC9.*K*^b-3; ◇, IC9.*K*^b-3.

the H-2 phenotype was determined in four different assays: (1) cytofluorography; (2) cellular radioimmunoassay; (3) lysis by alloreactive H-2-specific CTL; and (4) immunoprecipitation with monoclonal H-2 antibodies. All tests yielded identical results, but for the sake of brevity only a typical cellular radioimmunoassay using monoclonal H-2 antibodies and ¹²⁵I-labelled Protein A and a representative selection of transfected cell lines is presented.

Parental IC9 cells or IC9 transfected with *neo*^r alone express only the D^b molecule, whereas IC9 cells transfected with *H-2* genes also synthesize the K^b- or K^k antigens (Table 1). Correspondingly, parental IE7 cells or IE7 transfected with *neo*^r alone carry only D^b and D^k, whereas *H-2*-transfected IE7 also express K^b or K^k antigens. K^b- or K^k-positive cells were obtained only after transfection with the appropriate *H-2K* genes and never after subcloning of parental tumour cells or after transfection with *neo*^r alone (>500 colonies tested).

Because the intention of the work was to study the influence of *H-2* expression on interaction of the tumour cells with a syngeneic host, it was important to establish that the transfected K^b and K^k molecules are not modified in some way, for example by intragenic recombination of the transfected *H-2* gene with the host genome. Neither serological analysis with >20 monoclonal antibodies against distinct epitopes¹³ on the H-2 molecules (data not shown) nor two-dimensional gel electrophoresis revealed any abnormalities (Fig. 1). The K^b spots immunoprecipitated from K^b-transfected IC9 cells (marked with a circle) are identical to those obtained from a K^b-positive cell line of C57BL/6 origin, EL4. In addition, Northern blot analysis of *H-2K* end-specific messenger RNA, which is found in IC9 and IE7 cells only after transfection with *H-2K* genes, did not show any unusual H-2-specific mRNA (E. Meyer and G.J.H., unpublished). It is not yet clear why the parental IC9 and IE7 cells fail to express their H-2K antigens. That the respective *H-2K* genes are present in these tumours is shown by genomic Southern blot analysis with an H-2K-specific probe (E. Meyer and G.J.H., unpublished).

Two points can be made from these findings. First, transfection with cloned *H-2* genes can be used to restore H-2

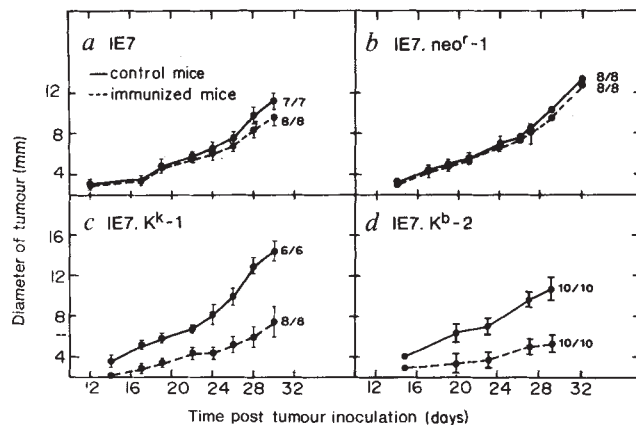


Fig. 3 *In vivo* immunogenic properties of IE7, parental and *H-2K*-transfected cells. (C3H.eB×C57BL/6J)F₁ female mice, aged 2 months, were inoculated with: *a*, 1×10^6 IE7 parental tumour cells; *b*, with IE7 cells transfected with *neo*^r gene only; *c*, with *H-2K*^b-transfected IE7 cells; *d*, with *H-2K*^b-transfected IE7 cells. One week later, the tumour-inoculated leg was amputated as described in Table 2 legend (immunized mice). Mice were then re-inoculated i.f.p. with 10^4 tumour cells of the immunizing origin. (Control mice were amputated as well.) Tumour growth in the immunized and control mice were measured using a caliper. Growth curves represent the mean tumour diameter of all mice inoculated $\pm$ s.e.m., including the diameter of legs of mice that did not develop tumours. Mean diameter of legs of control mice is 2 mm. The numbers designated above each growth curve represent the proportion of mice that developed a tumour.

expression in cells that have lost expression of their H-2 antigens. Second, there is no evidence for an alteration of the H-2 molecules encoded by the transfected genes.

Metastatic properties

Tumorigenicity and metastatic potential of the *H-2K*-transfected tumour cells was investigated by inoculation of 10^4 – 10^6 tumour cells into the hind foot pad of syngeneic (C3H×C57BL/6)F₁ mice. Expression of either K^b or K^k antigens on the surface of IE7 cells resulted in loss of their metastatic potential (Table 2). IE7 cells transfected with the *neo*^r gene alone retained the metastatic properties (for example, cell line IE7.*neo*^r-1). Consistent results were obtained with nine additional IE7 clones transfected with *neo*^r alone and with five additional IE7 lines derived from transfection with K^b or K^k genes (data not shown). In contrast to lack of metastasis in normal mice, the *H-2K*-transfected IE7 lines were found to metastasize in irradiated (C3H×C57BL/6)F₁ mice (Table 2). For irradiation, 550 rad were used, a dose that strongly reduces host immune reactivity. The findings suggest that the immune system of the host controls the ability of transfected cells to metastasize. Transfection of the non-metastatic IC9 cells with *H-2K* genes did not change their inability to metastasize in normal mice (Table 2). However, like transfected IE7, these cells were able to metastasize in irradiated mice.

Simultaneously, we tested whether the tumorigenic properties of the *H-2K*-transfected cells were also affected. We inoculated (into the foot pad) 10^4 transfected IE7 cells into syngeneic (C3H×C57BL/6)F₁ mice and recorded tumour growth (Fig. 2). K^b-positive IE7 cells (IE7.*K*^b-3) were found to grow significantly slower than the control IE7 cells (Fig. 2*a*). In fact, only three out of six mice developed a tumour. The transfected IE7 cells growing *in vivo* were still K^b-positive when analysed for H-2 expression (not shown) and, therefore, the tumour growth cannot be attributed to loss of K^b. In contrast, IE7 cells transfected with K^k, (IE7.*K*^k-1) or with *neo*^r alone (IE7.*neo*^r) grew like parental IE7 in all mice inoculated (Fig. 2*a*). Similar growth patterns were obtained with four other K^b, two K^k and nine *neo*^r-transfected IE7 cell lines and when different doses of transfected IE7 cells were injected (10^5 , 10^6 cells per mouse; data not shown). Thus, expression of K^b antigens on IE7 cells

decreased their tumorigenic properties. In irradiated mice, K^b -transfected IE7 grew like parental cells (Fig. 2b), suggesting that the immune system of the host also controls growth of the local tumour. The fact that IE7 cells transfected with K^k or K^b genes are both non-metastatic but manifest different growth rates suggests that expression of H-2K antigens on the tumour cells blocks metastasis irrespective of the growth kinetics of the tumour.

The tumorigenic properties of the non-metastatic IC9 clone were also affected by transfection with $H-2K$ genes. K^b -positive IC9 cells were found to grow more slowly in syngeneic F_1 mice than in parental IC9 or IC9 transfected with neo^r alone (Fig. 2c). In fact, cells of the transfected IC9. K^b -3 line were rejected in five out of six mice (Fig. 2c). For other K^b -transfected IC9 lines, the percentage of rejection was in general lower and varied between 5 and 20%. The higher rate of rejection of IC9. K^b -3 compared with other K^b -transfected IC9 lines seems to correlate with the amount of K^b expressed on these cells (see Table 1). The K^b -transfected IC9 growing in mice still expressed H-2K^b (not shown). Similar growth curves were recorded when 10^5 and 10^6 cells of IC9 transfected cells were inoculated, but the percentage of mice without a tumour was lower than with 10^4 injected cells (data not shown). All transfected IC9 lines were able to

grow like parental IC9 in irradiated mice (Fig. 2d). The results presented so far indicate that transfection of IC9 and IE7 cloned cells with $H-2K$ genes leads to the abolition of metastatic competence and a decrease in tumorigenicity. Our experiments with irradiated mice suggest that the immune system is involved in reduction of metastasis and tumorigenicity.

Immunogenic properties

We next tested whether the reduction in metastasis in $H-2K$ -transfected IE7 cells is associated with increased immunogenicity. F_1 mice were immunized with transfected tumour cells and then challenged with homologous tumour cells (Figs 3, 4). IE7 parental cells (Fig. 3a) and IE7 cells transfected with neo^r gene only (Fig. 3b) showed the same growth rate in immunized and control mice and, therefore, do not seem to be immunogenic. In contrast, immunization with IE7. K^k -1 (Fig. 3c) or with IE7. K^b -1 cells (Fig. 3d) led to retarded growth of the corresponding tumour cells. In agreement with previous observations¹⁰, the non-metastatic parental IC9 cells proved to be slightly immunogenic (Fig. 4a). However, expression of K^b on transfected IC9 cells led to a drastic increase in immunogenicity, resulting in complete rejection in respectively immunized mice (Fig. 4b-d).

Table 2 Metastatic potential of H-2K-transfected IE7 and IC9 cells in normal and irradiated syngeneic (C3H×C57BL/6) F_1 mice

Tumour	H-2 phenotype*				Mice inoculated	Weight of lungs (mg)	No. of nodules in lungs
	D ^k	K ^k	D ^b	K ^b			
IE7 transfected lines							
IE7	+	—	+	—	Normal	397 ± 70	12, 200, 200, 200, 200
					Irradiated	460 ± 20	200, 200, 200, 200, 200
IE7. <i>neo</i> ^r	+	—	+	—	Normal	420 ± 15	200, 200, 200, 200, 200
					Irradiated	395 ± 40	200, 2000, 200, 200
IE7.K ^k -1	+	+	+	—	Normal	186 ± 8	0, 0, 0, 0, 2, 6, 8
					Irradiated	381 ± 15	200, 200, 200, 200, 200
IE7.K ^k K ^b -1	+	+	+	+	Normal	170 ± 3	0, 0, 2, 2, 4
					Irradiated	401 ± 20	200, 200, 200, 200, 200
IE7.K ^b -1	+	—	+	+	Normal	168 ± 8	0, 0, 0, 0, 0
					Irradiated	326 ± 45	200, 200, 200, 200, 200
IE7.K ^b -2	+	—	+	+	Normal	182 ± 10	0, 0, 0, 0, 0, 0
					Irradiated	345 ± 50	200, 200, 200, 200, 200
IE7.K ^b -3	+	—	+	+	Normal	180 ± 5	0, 0, 0, 0, 0, 3
IC9 transfected lines							
IC9	—	—	+	—	Normal	172 ± 16	0, 0, 1, 3, 9, 200
					Irradiated	255 ± 52	3, 200, 200, 200
IC9.K ^b -1	—	—	+	+	Normal	162 ± 6	0, 0, 0, 0, 0, 5
					Irradiated	270 ± 8	200, 200, 200, 200
IC9.K ^b -2	—	—	+	+	Normal	154 ± 4	0, 0, 0, 0, 0
					Irradiated	283 ± 5	200, 200, 200, 200, 200
IC9.K ^k -1	—	+	—	+	Normal	171 ± 11	0, 0, 0, 0, 0

A single-cell suspension of 10^5 neo^r - and $H-2K$ -transfected IE7 and IC9 cells were inoculated intra footpad (i.f.p.) into intact and sublethally irradiated (550 rad Co) (C3H×C57BL/6) F_1 mice. When the tumour diameter reached a size of 8–10 mm, the tumour-bearing leg was amputated using a 3/0 silk tie, which was ligated beyond the iliofemoral joint of the hind leg to prevent bleeding. In each experimental group, mice were killed 21 days post-amputation, and their lungs removed and assessed for metastatic load by weight and by number of nodules counted after fixation in Bouin's solution. Without excision of the primary tumour the number of metastatic nodules is much lower²⁹. Corresponding results were obtained with both protocols, but only data generated with the excision protocol are presented. Other independent experiments yielded identical results (not shown).

* H-2 phenotype according to results presented in Table 1.

Table 3 Generation of CTL specific for transfected tumours in (C3H×C57BL/6) F_1 mice

Immunization	Target cells (% specific lysis)						EL4	L929
	IC9	IE7	(IC9. K^b -3)	(IE7. K^b -1)	(IC9. K^k -1)	(IE7. K^k -1)		
IC9	0	0	0	0	0	0	0	0
IE7	0	0	0	0	0	0	0	0
(IC9. K^b -3)	0	0	45	28	0	0	0	0
(IE7. K^b -1)	0	0	40	35	0	0	0	0
(IE7. K^k -1)	0	0	0	ND	ND	59	0	5

(C3H×C57BL/6) F_1 mice were immunized three times at weekly intervals with 10^6 tumour cells irradiated with 3,000 rad. After 7–10 days, spleen cells were restimulated *in vitro* with the same tumour cells irradiated with 10,000 rad. Cytolytic activity was determined at different effector-to-target ratios against ⁵¹Cr-labelled target cells including EL4 (H-2^b) and L929 (H-2^k) as controls. Data show per cent specific lysis obtained with an effector/target ratio of 50:1. ND, not determined.

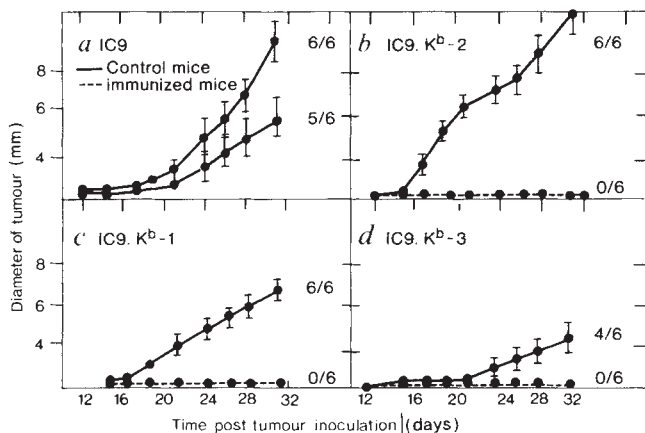


Fig. 4 *In vivo* immunogenic properties of IC9 parental and $H\text{-}2K^b$ -transfected cells. Syngeneic F_1 mice were immunized against: a, IC9 parental cells and, b–d, against $H\text{-}2K^b$ -transfected IC9 cells. Immunization and the immunogenic properties of the different tumour cells were followed as described in Fig. 2 legend.

In conclusion, expression of H-2K antigens on IC9 and IE7 cells confers highly immunogenic properties on these cells.

Generation of cytotoxic T lymphocytes

Spleen cells from syngeneic ($C3H \times C57BL/6$) F_1 mice immunized with irradiated tumour cells were restimulated *in vitro* and their cytotoxic activity was determined against a panel of target cells (Table 3). Immunization with parental IC9 or IE7 cells did not lead to generation of CTL. In contrast, specific Thy-1.2⁺ CTL were obtained, after immunization with K^b -transfected IC9 or IE7 cells. These CTL lysed only K^b -transfected IC9 and IE7 but not parental IC9 or IE7 cells, or EL4, an unrelated K^b -positive tumour. The findings suggest that the CTL can recognize an unknown tumour-associated antigen on IC9 or IE7 only in conjunction with K^b . Likewise, K^k -restricted CTL were found after immunization with K^k -transfected IE7. The fact that CTL induced with K^b -transfected IC9 can also lyse K^b -transfected IE7, and vice versa, indicates that both IC9 and IE7 carry the same tumour-associated antigen.

Discussion

The data presented here suggest that manipulation by gene transfection of H-2K expression on ($H\text{-}2^b \times H\text{-}2^k$) F_1 mouse-derived T10 sarcoma cells leads to changes in the malignant properties of these cells. Thus, the H-2K-negative and highly metastatic IE7 cells lost their capacity to metastasize when made to re-express H-2K antigens by $H\text{-}2K$ gene transfection. Moreover, K^b -transfected IE7 or IC9 cells manifested a decrease in their tumorigenic properties. The crucial question is whether the modulation of tumour growth does indeed result from the *de novo* expression of H-2K antigens. The most convincing argument is that all IE7 clones expressing only the *neo*^r gene still metastasize and grow *in vivo* like the parental IE7 cells. Similarly, all IC9 clones transfected with *neo*^r alone grow like the non-metastatic parental IC9.

IE7 cells transfected with either $H\text{-}2K$ or parental and transfected IC9 metastasized when inoculated into sublethally irradiated mice, suggesting that in the case of these tumour cells the immune system is involved in preventing metastasis formation. Indeed, the efficient immunization with H-2K-positive IE7 and IC9 cells (Figs 3, 4) and the induction of CTL by $H\text{-}2K$ -transfected cells (Table 3) demonstrates that the *de novo* expression of H-2K converts the tumours into highly immunogenic cells. The resulting CTL lysed only H-2K-positive, but not parental, cells. These findings indicate not only that the presence of H-2K antigens on the tumour cells is required for induction of immunity, but also that H-2K is part of the target structure recognized by effector cells.

The results presented here are consistent with many reports showing that CTL raised against foreign cellular antigens can be preferentially restricted by the H-2K or the H-2D molecules depending on the antigen and H-2 haplotype involved¹. Thus, it is conceivable that in the case of the IC9/IE7 tumour system, the D^b and D^k molecules fail to act as H-2 restriction elements for T-cell recognition. In contrast, the putative tumour associated antigen on IE7 cells, the nature of which is unknown, can be efficiently recognized in conjunction with the transfected K^b or K^k , resulting in the elimination of disseminating metastatic cells. As only expression of K^b but not K^k leads to retarded growth of the primary tumour, it seems that K^b renders the cells more immunogenic than K^k . However, the resulting K^k -restricted immune response seems to be strong enough for elimination of metastasizing K^k -transfected IE7 cells, possibly because the disseminating cells are more accessible than the locally growing tumour. In addition to the potential failure of H-2D molecules to function as H-2 restriction elements, accumulating data suggest that H-2D antigens may play an active role in the prevention of immune responses^{14–17}. In fact, recent experiments suggest that the D^k molecule on IE7 cells exerts suppressogenic effects on the host response⁹. Other non-immunological effects of H-2 antigens cannot be excluded at this point.

The frequently observed absence of class I MHC antigens on tumours^{2,3,18,19} may have important implications for the immune surveillance of neoplastic cells. This notion has been substantiated here and by other recent reports^{20–22}. While this manuscript was in preparation, Hui *et al.*²³ reported that restoration of H-2K^k expression by gene transfection led to reduced tumorigenicity of a non-metastatic AKR thymoma, which is in agreement with our observations on the solid T10 sarcoma. The functional importance of class I MHC antigens in the animal systems described above may also have implications for the phenotypic expression of malignancy of human neoplasms. Indeed, there is some recent evidence that the malignancy of certain human tumours seems to be correlated with expression of HLA class I antigens^{24,25}.

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RS Ophiuchi — first radio detection of a recurrent nova outburst

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Recurrent novae and classical novae belong to the cataclysmic variable class of objects. Of these, only classical novae outbursts have been hitherto observed at radio wavelengths, and then no earlier than 50 days after the outburst. Here we report the first radio detection of an outburst from a recurrent nova (RS Ophiuchi). The observations begin only 18 days after the optical maximum and show a radio 'light' curve which is different from that of classical novae. The brightness temperature of $\sim 10^7$ K, calculated for this event, greatly exceeds the 10^4 K seen in classical novae, and suggests a non-thermal origin for the radio emission from RS Ophiuchi. Although the later emission has a classical $(t - t_0)$ 'light' curve, the radio emission mechanism appears not to turn on until 14 days after the optical nova and takes a further 10 days to reach the $(t - t_0)$ curve. Analogies may be drawn between the radio development of RS Ophiuchi and that of young super-novae.

In both recurrent and classical novae, the outbursts may result from thermonuclear runaway on the surface of a white dwarf in a close binary system, which leads to a rapid increase in luminosity and mass ejection¹. Based on this model there are two major differences between recurrent and classical novae. First, the secondary component of the binary system in recurrent novae is thought to be a late-type giant rather than a main-sequence dwarf as in classical novae. Second, the inter-outburst timescale is tens of years rather than 10^4 – 10^5 years as predicted for classical novae; for example, the recurrent nova RS Ophiuchi has previously undergone very similar outbursts in 1898, 1933, 1958 and 1967 (ref. 2).

Following the nova outburst reported by Morrison³ on 26 January 1985, observations were made with the new Jodrell Bank Mk IA, Mk II 400-MHz bandwidth interferometer system operating at 5 GHz (S.P. and R.J.D., in preparation). This instrument has a baseline of 425 m, giving a lobe spacing of ~ 30 arc s at 5 GHz. At this frequency, the 4-arc min beam of the Mk IA telescope results in a typical confusion level of 1 mJy. The first flux density measurement of RS Ophiuchi was obtained on 13 February, only 18 days after the outburst. This first detection yielded a flux density of 23 ± 3 mJy and subsequent observations on 15 February revealed an increase in flux density⁴.

Phase measurements made with the interferometer allow the position of the radio emission to be established to within ± 2 arc s in right ascension and ± 4 arc s in declination; within these limits the radio position was found to be consistent with the reported optical position of the nova. Figure 1 shows observations of the radio 'light' curve at 5 GHz for RS Ophiuchi between 13 February and 5 March. Further observations were not possible because of telescope scheduling considerations. The flux density calibration of these data is based ultimately on observations of 3C286, which is assumed to have a flux density of 7.30 Jy at 5.0 GHz (ref. 5), with the gain-elevation dependence of the interferometer being obtained from observations of many different radio sources. For all these observations, a referencing technique was used with the instrument observing a nearby calibrator source for a short time before moving to the field of interest. Using such a procedure, the flux density of the nova is accurately related to that of the source 1741-038 and this is reflected in the relative error bars in Fig. 1. However, the flux density of 1741-038 is not accurately known and so this has been related to 3C286. This latter calibration introduces a further systematic error of $\sim 5\%$ into the flux density scale.

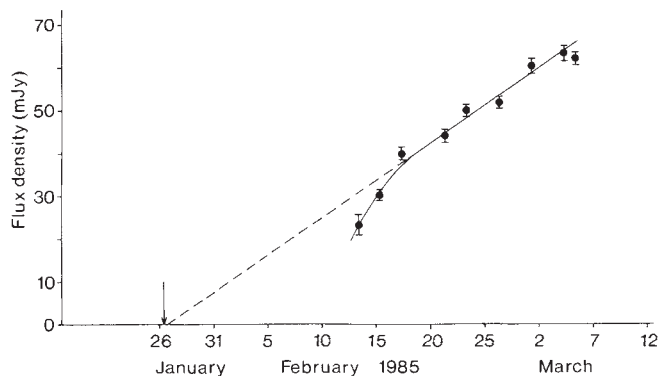


Fig. 1 Radio 'light' curve for the recurrent nova RS Ophiuchi measured at 5 GHz with left-hand circular polarization. The vertical arrow indicates the time of the optical outburst.

Figure 1 clearly shows two phases in the radio development of the nova. Initially there is a rapid increase in flux density at a rate of approximately 4 mJy per day until 18 February; this then gives way to a slower linear increase which projects back to the time of the initial outburst (t_0), giving a $(t - t_0)$ dependence with a constant of proportionality of 1.7 mJy per day. This $(t - t_0)$ dependence is characteristic of the classical radio novae observed by Hjellming *et al.*⁶. The initial rapid rise indicates that the radio emission 'turned on' about 14 days after the nova explosion, but after a further 10 days it reached the $(t - t_0)$ curve. Thus, although the radio emission was 14 days late in turning on, after ~ 24 days the curve proceeds as if the emission had started at t_0 .

Analogies may be drawn between the radio development of RS Ophiuchi and that of Type II supernovae. In the latter case, the non-thermal radio emission is thought to arise from the interaction of an ejected shell with the pre-outburst wind of the late-type supergiant progenitor⁷. Similarly, RS Ophiuchi is thought to eject $\sim 10^{-5} M_{\odot}$ of material at $\sim 1,000$ km s⁻¹ into pre-existing circumstellar material⁸ which may be associated with a wind from the giant secondary of a binary system.

If we assume a distance of 2 kpc (ref. 9) and an average expansion velocity of 10^3 km s⁻¹, we calculate a brightness temperature of 10^7 K for the period of our observations. Initial measurements with the Very Large Array indicate a nearly flat spectrum (R. M. Hjellming, personal communication). Classical novae⁶ exhibit thermal radio emission, typically with a temperature of 10^4 K, and during the optically thick, rising phase of the light curve have a spectral index of $\sim +1.5$. These differences lead us to suggest a non-thermal origin for the radio emission in RS Ophiuchi. As the $(t - t_0)$ dependence is similar to that of a classical nova, albeit with a different constant of proportionality, these observations suggest that both the optical depth and dynamics of the shell after 24 days may be similar for the two phenomena, even though the radio emission mechanism may differ. The subsequent radio evolution of this system is clearly of considerable interest.

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Lower stratosphere trace gas detection using aircraft-borne active chemical ionization mass spectrometry

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Acetaldehyde and acetone are expected to be formed as trace gases in the free troposphere from photochemical conversion of non-methane hydrocarbons and to represent key precursors of peroxyacetyl nitrate (PAN)¹⁻⁵. PAN is important as a potential organic reservoir¹⁻⁵ for odd N and thus as a vehicle for odd N transport in the troposphere or even from the troposphere to the stratosphere. As PAN suffers from thermal decomposition in the lower troposphere, its transport into the stratosphere relies on its formation in the upper troposphere where temperatures are sufficiently low to prevent thermal decomposition. Neither PAN nor its key precursors, however, have been observed in the free troposphere and it is difficult, therefore, to assess the potential impact of non-methane hydrocarbons on upper tropospheric and stratospheric ozone¹⁻⁵. An indication of the presence of acetone around the tropopause level was first obtained recently from ambient positive-ion composition measurements⁶. Here, we report on a search for these trace gases and other species with large proton affinities in the tropopause using aircraft-borne active chemical ionization mass spectrometry (ACIMS), a novel method for atmospheric trace gas detection.

The ACIMS method developed in our laboratory will be described in detail elsewhere^{7,8} and only a brief outline is given here. Detection by ACIMS of molecules B possessing large proton affinities or gas phase basicities relies on their reaction with $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions in



Usually⁸ proton transfer reactions (1), if sufficiently exothermic, occur on every collision and have a temperature-independent rate coefficient of $\sim 2\text{--}5 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ depending on the dipole moment of B. The ACIMS apparatus (Fig. 1) works like a flow reactor where ambient air is sampled by an air intake on top of the aircraft's fuselage and then the air stream is passed through a flow tube and directed to the sampling cone of a cryogenically pumped quadrupole mass spectrometer. Ultimately, the air stream leaves the aircraft's fuselage through an outlet tube. Precursor ions $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ are formed by a high-frequency glow-discharge ion source inside the flow tube, 70 cm upstream of the sampling cone that carries the inlet orifice through which ions enter the mass spectrometer. The time t_R for which ions reside in the reaction zone between the ion source and the sampling cone, being typically 17 ms, can be measured precisely by pulsing the ion source and measuring the ion arrival time. By measuring the flux ratio $F(\text{HB}^+)/F(\text{H}_3\text{O}^+)$ for precursor

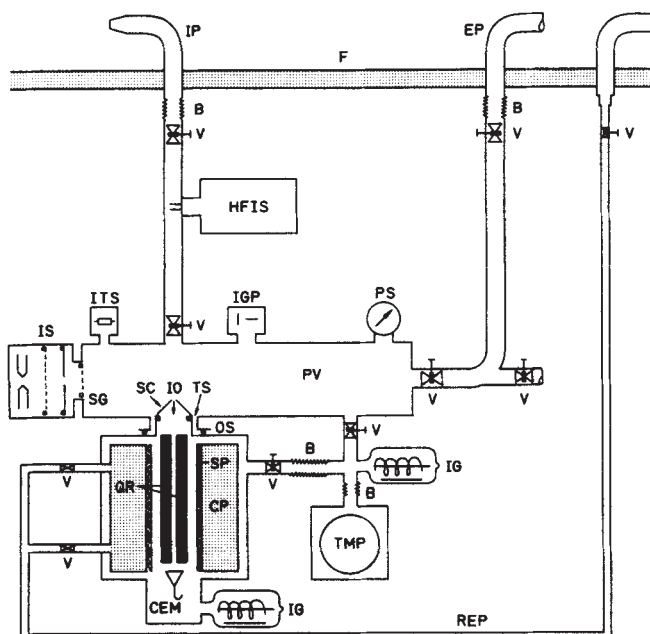


Fig. 1 Schematic representation of the aircraft-borne chemical ionization mass spectrometry apparatus. F, fuselage; IP, intake pipe; EP, exit pipe; V, valve; HFIS, high-frequency glow-discharge ion source; IS, electron impact ion source; ITS, temperature sensor; IGP, ion getter pump; PS, pressure sensor; SG, sampling grid; PV, probing vessel; SC, sampling cone; TS, Teflon seal; IO, inlet orifice; OS, O-ring seal; QR, quadrupole rods; CP, cryopump; SP, sorption panel; B, below; IG, ionization gauge; TMP, turbomolecular pump; CEM, channel electron multiplier.

and product ions with the mass spectrometer, the [B] can be obtained using expression

$$[\text{B}] = \frac{1}{k t_R} \ln \left(\frac{F(\text{HB}^+)}{F(\text{H}_3\text{O}^+)} + 1 \right) \quad (2)$$

which follows from integration of the kinetic equations for product and precursor ions.

Taking the above k and t_R and an ion flux ratio of 0.001 which can easily be measured, a conservative estimate for the typical detection limit of $\sim 3 \times 10^7 \text{ cm}^{-3}$ corresponding to a volume-mixing ratio of $\sim 4 \times 10^{-12}$ or 4 p.p.t.v. (parts per trillion by volume) at $\sim 11,000$ altitude is obtained. The uncertainty in [B] is determined primarily by the uncertainty in k , which may be as low as $\pm 30\%$ in laboratory measurements. Typical uncertainties for t_R and the ion flux ratio are both $\pm 20\%$. Taking a typical uncertainty for k of $\pm 50\%$, an estimate of the overall uncertainty for [B] of $\pm \sim 57\%$ is obtained. The precision of [B], which is not affected by the uncertainty in k , is $\pm \sim 28\%$.

The first flight of the novel ACIMS instrument was on 24 February 1984 over the FRG, reaching a maximum altitude of

Table 1 Concentrations and volume mixing ratios for trace gases measured in flight F5 at 11,300 m

Molecule	PA (kcal mol ⁻¹)	k (10 ⁹ cm ³ s ⁻¹)	Concentration (10 ⁻⁸ cm ⁻³)	VMR (10 ¹²)	Other data	Ref.
CH ₃ CN	187	3.2	2.0	36	2-4, 20,000 m	10-12
					56, Ground	13
(CH ₃) ₂ CO	193.6	2.9	1.4	25	≥ 20 , 11,300 m	6
					(F5 PACIMS)	
					500, Ground	16
					15, 9,000 m (Model)	1
NH ₃	200.4	1.8	≤ 4.0	≤ 73	≤ 0.050 , 11,300 m	6
					(F5 PACIMS)	
					600-2,000, 11,000 m	14
					600, 4,000 m	15
					500-20,000, Ground	1
CH ₃ CHO	185.4	2.3	≤ 0.11	≤ 2.0	3.0, 9,000 m (Model)	1
					7.0, 15,000 m (Model)	1

PA, proton affinities (see ref. 9); VMR, volume mixing ratios. References are for other data.

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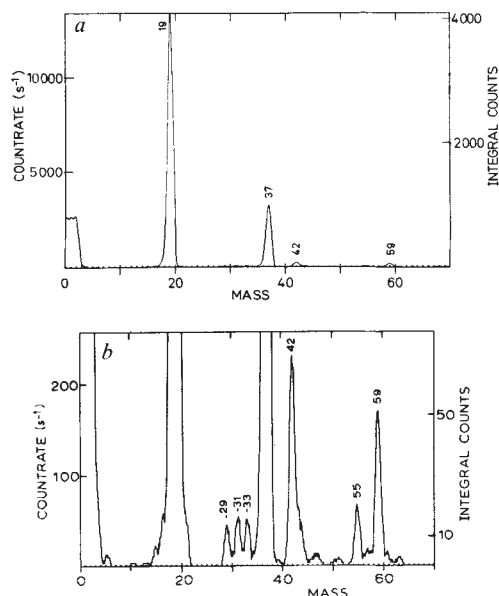


Fig. 2 *a*, Positive-ion ACIMS mass spectrum obtained at 4,300-m altitude. Panel *b* is enlarged in the *y*-direction for visualization of minor ion peaks. The signal at masses 0–4 does not represent a mass peak but is a reduced total ion signal occurring at very low radiofrequency-amplitude of the mass filter. Integral counts denotes the total number of ion counts per mass peak.

11,300 m, well above the local tropopause levels (9,750–10,360 m) and cloud-top levels (5,000–8,000 m). ACIMS measurements were taken only at 11,300 m and only for a short period of time (15.19–15.33 LT) and the remaining flight time was spent for ambient ion composition measurements⁶.

A typical positive-ion ACIMS spectrum, obtained in 40 s at 11,300 m, is shown in Fig. 2. Besides the precursor ions $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ (masses 19, 37, 55), it contains several product ion peaks of which 42 and 59 are most prominent. Like H_3O^+ , the most prominent member of the $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ family, these ions must be naked 'cores' of the type HB^+ , with B possessing a large proton affinity (typically $> \sim 175 \text{ kcal mol}^{-1}$). Therefore, the most probable candidates are protonated acetonitrile (42) and protonated acetone (59); no significant peaks attributable to protonated acetaldehyde (45) and protonated ammonia (18) occur.

Taking measured rate coefficients k (Table 1), concentrations (acetonitrile and acetone) and upper limits (ammonia and acetaldehyde) were obtained from equation (2). A compilation of our measured trace gas abundances is given in Table 1 together with other data. (Data for additional trace gases detected by the present ACIMS experiments are given elsewhere.)

Our present acetonitrile abundance of 36 p.p.t.v. at 11,300 m is similar to the average ground-level value of 56 p.p.t.v. obtained by Snider and Dawson¹⁵ (Fig. 3), which suggests that acetonitrile has a sufficiently long lifetime to allow for efficient vertical mixing in the troposphere (typical timescale: several days). This is consistent with recent estimates of CH_3CN -lifetimes against reaction¹⁸ with OH and washout¹⁹ being about 1–2 years in the troposphere. In contrast, middle stratosphere acetonitrile mixing ratios as obtained from balloon-borne PACIMS (passive chemical ionization mass spectrometry) measurements^{11,17} are much lower than our present value at 11,300 m, which is somewhat surprising in view of the relatively slow reaction of CH_3CN with OH, the only presently known CH_3CN -sink in the lower stratosphere. A one-dimensional photochemical model calculation of CH_3CN by Brasseur *et al.*¹⁸ (also shown in Fig. 3) considering reaction with OH as the only CH_3CN -sink requires a tropospheric CH_3CN -volume mixing ratio of only 10 p.p.t.v. in order to reproduce the middle stratosphere observations. Compared to this value our present measured value of 36 p.p.t.v. and the average ground-level value of 56 p.p.t.v. are much larger.

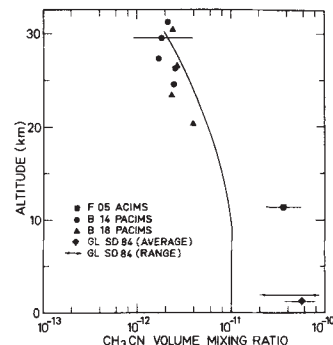


Fig. 3 Comparison of measured acetonitrile volume mixing ratios as obtained from aircraft-borne PACIMS^{11,17} and ground-level (GL) condensation collection¹³; typical error bars for the various analytical methods are indicated. Also shown is a one-dimensional photochemical model calculation for CH_3CN by Brasseur *et al.*¹⁸ considering reaction with OH as the only CH_3CN -sink.

The reason for this discrepancy is presently not known. Possibilities include an additional lower stratosphere CH_3CN -sink or a non-uniform tropospheric CH_3CN -distribution with lower abundances in equatorial regions where tropospheric air primarily enters the stratosphere. However, it should be kept in mind that the stratospheric distribution of CH_3Cl , which is also removed by reaction with OH at a rate similar to CH_3CN and which is practically uniformly mixed in the troposphere, is not well reproduced by one- and two-dimensional model calculations. Usually stratospheric CH_3Cl is overestimated by the models²². Concerning additional CH_3CN -sinks in the lower stratosphere the possibility of CH_3CN -removal by ion processes may also be considered in view of the fact that stratospheric positive ions contain CH_3CN . Upon ion-ion recombination of such CH_3CN -containing clusters, CH_3CN may be destroyed by reaction or be incorporated into aerosols which may eventually result from ion-ion recombination^{20,21}. However, very little is known about the products of ion-ion recombination and thus the possibility of CH_3CN -removal by ions remains speculative.

Compared with acetonitrile the situation for ammonia is entirely different. Here, the upper limit of 73 p.p.t.v. is much lower than the upper troposphere and lower stratosphere measurements yet made. Only two measurements at $\sim 11,000 \text{ m}$ have been reported (2 p.p.b.v., March 1979; 0.6 p.p.b.v., August 1979)¹⁴ using a remote infrared heterodyne radiometer technique. Our value is also much lower than the chemical filter data (0.6 p.p.b.v.) obtained¹⁵ at $\sim 4,000 \text{ m}$. Again, more free tropospheric measurements covering a range of meteorological conditions are needed before conclusions on ammonia transport and removal can be reached.

We next discuss acetone and acetaldehyde. The present acetone abundance of 25 p.p.t.v. is higher than the model predictions of Singh and Hanst¹, being 15 p.p.t.v. (9,000 m) and 3 p.p.t.v. (15,000 m). By contrast, the upper limit described here for acetaldehyde of 2 p.p.t.v. is somewhat lower than the model predictions¹ (3 p.p.t.v., 9,000 m; 7 p.p.t.v., 15,000 m). If the kinetic data used by Singh and Hanst¹ are correct, PAN formation at 11,300 m from acetone would be at least 10 times more efficient than from acetaldehyde. A further implication is that the production of acetone would be much larger than predicted by the model (considering only propane as a precursor of acetone). Thus, either the propane level was higher than assumed or additional non-methane hydrocarbon species contribute significantly to acetone formation. It is also conceivable that the observed increase in acetone level is associated with jet aircraft exhaust, a possibility that deserves further attention. Alternatively, the model may underestimate the lifetime of acetone in the troposphere.

Clearly, more measurements, in particular height profiles, of acetone, and eventually also acetaldehyde, in the free troposphere and lower stratosphere are needed to elucidate the details of PAN formation in this part of the atmosphere. The ACIMS method has the potential to contribute significantly to an

improved understanding of the photochemistry of non-methane hydrocarbons in the free troposphere and lower stratosphere.

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An Antarctic ice core reveals atmospheric CO₂ variations over the past few centuries

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When snow is transformed into ice (firnification process) by sedimentation near the surface of an ice sheet, some of the atmospheric air is trapped in the inter-grain spaces which are progressively isolated from the surrounding atmosphere. The resulting material is an air-tight bubbly glacial ice. By analysing the air extracted from the bubbles found in the ice, it is possible to determine the air composition and thus its CO₂ content for the period during which the air was trapped. We aim here to provide the most direct evidence obtained so far for the background atmospheric CO₂ concentrations over the centuries preceding the recent anthropogenic perturbation due to the industrial revolution of the past century. This background level is important for assessing both the origin and the climatic response of the anthropogenic perturbation to the atmospheric CO₂ (see ref. 1). Our results, obtained from the analysis of the air trapped in the bubbles of an

Antarctic ice core, indicate that the background level could have been as low as 260 p.p.m.v. (parts per million by volume) before the major anthropogenic influence and suggest that the so-called 'pre-industrial' CO₂ level was not constant over the few hundred years preceding the nineteenth century.

Although several parameters, such as snow melting at the surface of the ice sheet, may obscure the record, CO₂ results representing the atmosphere can be obtained with a good degree of confidence by choosing appropriate sites, cores and gas extraction methods (see ref. 2). In particular, the best conditions are found using unfractured ice cores taken in the dry snow zones where no melting occurs (the mean annual air temperature of these zones is generally below -25°C). The ice record of atmospheric CO₂ against time is, furthermore, 'smoothed' because each ice layer contains bubbles which closed off at different times during the firnification. Because the firnification rate increases with the rate of snow accumulation at the surface, a low smoothing effect is expected in high-accumulation sites. For this study, the smoothing must be as low as possible to detect, with sufficient definition, the possible atmospheric CO₂ changes over a few centuries. In short, we are looking for a good core taken in a cold site without summer melting and with a high accumulation rate.

Such requirements were fulfilled by a 203-m-long ice core drilled in 1980-81 at the station D57 (68°11'S, 137°33'E) in East Antarctica. The ice from this core analysed for the present study was formed, according to an ice origin calculation, upstream from the drilling site in a zone where the mean annual surface temperature is ≤ -32°C (-32°C at D57) and the present-day measured snow accumulation rate is 45 cm of ice equivalent yr⁻¹ at D57 and highly variable upstream (M. Pourchet, personal communication). Taking into account the accumulation data and the conditions of gas enclosure, each air sample analysed could average between ~20 and 40 yr of atmospheric conditions.

For CO₂ measurements, we sampled eight depth levels between ~89 and 198 m below the surface (Table 1). For each level, between 16 and 50-cm lengths of the ice core were analysed after the ice samples (weighing between 23 and 46 g) had been cut into horizontal core layers ~2-4 cm thick. These ice samples were taken from the internal and unfractured part of the ice cores and cut in a cold room (at about -15°C) with a band saw. The whole experimental procedure, including all the steps between the extraction of the gas by grinding the ice under vacuum at -40°C and the gas chromatographic analysis, was performed using the Grenoble method described in ref. 3.

For each measurement, the analytical system is calibrated as described in ref. 3 and the experimental accuracy (with a 95% confidence limit) is evaluated from the standard deviation of the residuals corresponding to the calibration regression. This accuracy for the results presented here is in the range ±1.1-4.8%. Between 5 and 13 samples were processed for each depth level. The corresponding CO₂ results are shown in Table 2. The scattering of the CO₂ measurements for a given depth level is evaluated by calculating their standard deviation and lies in the range ±1.3-3.8% (Table 1). This scattering is smaller than, or

Table 1 D57 ice-core sample characteristics, mean CO₂ concentrations and mean ages of the gas

Depth below the D57 surface (m)	Length of ice core analysed (cm)	No. of samples analysed	Scattering of CO ₂ measurements (%)	Mean CO ₂ concentration (p.p.m.v.)	Mean age of the gas (AD)	
					a	b
89.3-89.6	24	7	2.4	288	1940	1890
102.4-102.6	19	7	3.8	266	1915	1830
124.8-125.0	17	6	1.3	261	1855	1710
133.0-133.7	16	5	2.8	259	1835	1670
141.3-142.0	50	13	2.6	264	1815	1630
161.6-161.8	16	7	2.0	268	1745	1520
181.1-181.3	17	6	1.3	272	1620	1420
197.2-197.5	24	6	3.4	271	1560	1340

The mean ages of the gas are calculated (see text) taking into account a constant rate of bubble enclosure between the densities 0.80 and 0.83 and ages of ice obtained from modelling the ice flow (a) and by assuming a constant accumulation rate of 18 cm of ice equivalent yr⁻¹ (b).

similar to, the experimental errors and can consequently be attributed to them. The mean CO₂ concentrations measured for each depth level are shown in Table 1.

Our calibration gas has been compared with standards prepared according to the WMO 1974 provisional scale by the Scripps Institution of Oceanography³ and the error due to the calibration gas on the absolute values of the CO₂ measurements is estimated to be $\leq \pm 1\%$. An absolute calibration of the complete procedure is, unfortunately, not possible because a standard ice enclosing bubble having a known composition is not available. Nevertheless, we recently simulated such a calibration by grinding artificial bubble-free ice surrounded by a volume of calibration gas equivalent to the volume of air trapped in the natural ice. The results indicate that the procedure does not change the CO₂ composition of the gas within experimental error. A similar conclusion has been reached after comparing CO₂ measurements on air from bubbles in polar ice performed in our laboratory and in the Physics Institute of Bern³. Nevertheless, more recent measurements made in Bern suggest that their results would have to be corrected by adding ~ 15 p.p.m.v. (ref. 4). A new comparison is in progress between the two laboratories to solve this uncertainty.

We will now evaluate when the air found in the bubbles was isolated from the free atmosphere. As pointed out above, the bubbles become isolated from the free atmosphere during the firnification process. Consequently, the gas at a given depth is younger than the ice itself. The bubble close-off process begins to be significant probably after the firn grains have been packed into the closest possible arrangement. This closest possible packing corresponds, according to Anderson and Benson⁵, to a firn density of ~ 0.55 . The enclosure process ends at the firn-ice transition, at a density of ~ 0.83 . Recent volume measurements of the closed bubbles in the firn at Siple Station, Antarctica, by Schwander and Stauffer⁶ indicate that 80% of the bubbles close off between the densities 0.795 and 0.83. Nevertheless, the observed occurrence of impermeable layers and the fact that the air, especially in the deeper part of the firn, could not be well mixed with the atmosphere, may isolate the air in the firn from the free atmosphere at shallower firn densities (and consequently at shallower depths). Furthermore, although the experimental method used by Schwander and Stauffer is the best available, we believe that this method risks breaking some bubbles near the sample surface. If this is the case, the method provides a minimum volume value of the closed bubbles, especially for the firn with relatively low densities. Also, the

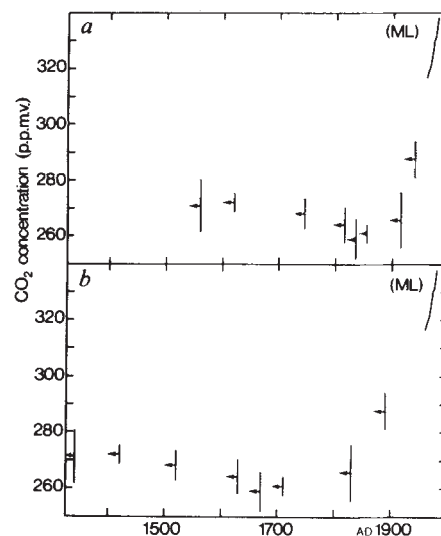


Fig. 1 Atmospheric CO₂ concentrations during the past centuries as deduced from the D57 ice-core analysis. The vertical lines indicate the scattering ($\pm 1\sigma$) of the measurements. The mean ages plotted were obtained using two different approaches (see text). *a*, Dating of the ice obtained by modelling the ice flow. *b*, Dating based on the Tambora horizon at a depth of ~ 48 – 50 m below the surface and on a constant snow accumulation rate for the whole period encompassed by the core. The arrows indicate qualitatively that the mean ages may be older if the air in the firn is isolated from the atmosphere before the firn reaches the density 0.80. ML, The atmospheric CO₂ record since AD 1958 at Mauna Loa¹⁰.

enclosure conditions could be different at D57 because the firnification parameters (temperature and snow accumulation rate) are not the same as at Siple Station. For these reasons we assume that: (1) the bubbles close off at a constant rate between the densities 0.80 and 0.83 which correspond, respectively, to depths of ~ 68 and 76 m below the surface at D57; and (2) the air can be isolated before the firn reaches the density 0.80. Taking into account the time of deposition of the snow at the surface of the ice sheet, that is, the age of the ice, and the time required to reach the densities 0.80 and 0.83, it is possible to calculate mean ages of the air samples we analysed. These gas ages are given in Table 1 and Fig. 1 for two different estimates of the age of the ice. They may be, in fact, older by several tens of years, as suggested by the arrows in Fig. 1, because the air can be isolated before the firn reaches the density 0.80. This uncertainty would be, in the case of D57, generally smaller than the uncertainty in the determination of the age of the ice.

For dating the ice, we have used two approaches. First, the depth-age relationship has been calculated (C. Ritz and D.R., unpublished data) from modelling the ice flow upslope from D57 by assuming a uniform vertical strain rate and horizontal ice velocities independent of the depth. The parameters required for the calculation include snow accumulation rate, horizontal ice velocity and ice thickness at the sites of snow deposition. The times required for the snow to reach the densities 0.80 and 0.83 have been obtained in a similar way. This dating depends essentially on snow accumulation data. Unfortunately, the accumulation rate pattern upslope from D57 is highly variable and poorly known. In this calculation, we take into account the limited accumulation rate data available: 45, 32, 4.4, 15, 34 and 28 cm of ice equivalent at D57 and 10, 15, 16, 20 and 33 km upslope from D57 respectively (M. Pourchet, personal communication). These data are representative of between 4 and 28 yr of present-day accumulation. The mean age of the gas corresponding to this approach is shown in Table 1 and Fig. 1*a*. The second approach takes into account two exceptionally marked sulphate horizons recorded at 48 and 50 m below the surface⁷. According to Legrand and Delmas (personal communication) (1) these two horizons are undoubtedly volcanic and have also been recorded in the Dome C and South Pole cores

Table 2 Results of the multiple CO₂ measurements performed at each depth level for the D57 ice core

Depth below the D57 surface (m)	CO ₂ concentrations with experimental accuracies (p.p.m.v.)		
89.3–89.6	275.5 $\pm$ 8	288.5 $\pm$ 8	281.5 $\pm$ 9
	289.5 $\pm$ 8	292 $\pm$ 7	294 $\pm$ 9
	293 $\pm$ 8		
102.4–102.6	251 $\pm$ 7	256 $\pm$ 6	266 $\pm$ 3
	270 $\pm$ 8	262 $\pm$ 6	276 $\pm$ 3
	279 $\pm$ 3		
124.8–125.0	258.5 $\pm$ 12	257.5 $\pm$ 10	260.5 $\pm$ 10
	267.5 $\pm$ 10	261 $\pm$ 8	261.5 $\pm$ 10
133.0–133.7	258 $\pm$ 8	247 $\pm$ 9	262 $\pm$ 9
	262 $\pm$ 4	266 $\pm$ 4	
141.3–142.0	252 $\pm$ 7	255 $\pm$ 7	254 $\pm$ 7
	272 $\pm$ 8	270 $\pm$ 13	267.5 $\pm$ 7
	264 $\pm$ 10	270.5 $\pm$ 11	263 $\pm$ 6
	267.5 $\pm$ 6	273 $\pm$ 12	263 $\pm$ 8
161.6–161.8	263 $\pm$ 4		
	264.5 $\pm$ 5	261.5 $\pm$ 6	273 $\pm$ 5
	263 $\pm$ 8	272 $\pm$ 11	276 $\pm$ 7
181.1–181.3	268 $\pm$ 9		
	274.5 $\pm$ 5	277.5 $\pm$ 10	268.5 $\pm$ 9
	270.5 $\pm$ 10	272.5 $\pm$ 8	269 $\pm$ 10
197.2–197.5	258 $\pm$ 8	264.5 $\pm$ 8	270.5 $\pm$ 8
	278.5 $\pm$ 8	284 $\pm$ 7	271 $\pm$ 7

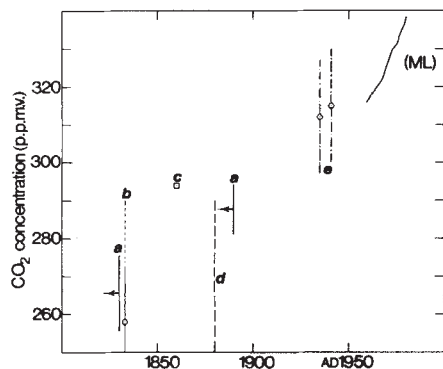


Fig. 2 Comparison between the CO₂ results from the D57 ice core and other estimates for the period since AD 1830. For the D57 results (a) only those ages in Fig. 1b for which we have most confidence for this time period are plotted. b–e, Estimates of past atmospheric CO₂ concentrations (discussed in refs 8 and 9) based on: b, the analysis of sub-surface ocean waters; c, a model calculation extrapolating from ML by assuming fossil-fuel combustion to be the only source of CO₂; d, the interpretation of some early chemical measurements of atmospheric CO₂; and e, preliminary analyses of archived solar spectra. ML is the atmospheric CO₂ record observed at Mauna Loa since AD 1958 (ref. 10).

which are independently datable with much more confidence than the D57 core; (2) the most likely candidate for one of these events is the Tambora eruption, AD 1815. This AD 1815 level at ~48–50 m below the surface would correspond to a mean accumulation rate of ~18 cm of ice equivalent yr⁻¹ during the past 165 yr. We have tentatively calculated (Table 1) and plotted in Fig. 1b what would be the mean age of the gas if the accumulation rate remained constant at this value for the whole time period encompassed by the core. The two approaches give important differences (from 50 to 220 yr) in the mean age of the gas for the levels measured in CO₂. This clearly demonstrates the large uncertainties in the dating of the gas trapped in the D57 core. Nevertheless, the sulphate horizons found at 48 and 50 m below the surface are an important constraint for the dating of the upper part of the core and consequently, for the youngest part of the CO₂ record, we have more confidence in the timescale shown in Fig. 1b.

Whatever the dating uncertainties, this first detailed CO₂ record over the past few centuries from ice-core analysis indicates (Fig. 1) that the atmospheric CO₂ concentrations were at a minimum some time between AD ~1650 and 1850, with a mean value of ~260 p.p.m.v. This value must be compared with the ~295 p.p.m.v. calculated for the pre-industrial level (AD 1860 and before) by extrapolating observed CO₂ values from 1958 to the present and assuming that fossil fuels were the only sources^{8,9}. The difference between the measured values from the D57 core and the results of this calculation strongly suggests that another important source has been active during the period preceding any significant use of fossil fuels, even if we have to correct systematically the measured values by +15 p.p.m.v. Taking into account the two youngest CO₂ levels plotted on Fig. 1b against the age that we favour for this part of the record, the main origin of this source may very well be the agricultural 'revolution' which began seriously to modify the biosphere during the past century.

Another interesting feature of the D57 core is, as shown in Fig. 1, that a mean decrease of the order of 10 p.p.m.v. may have occurred over a period of a few centuries before AD 1800. This fluctuation may reflect natural changes in the biospheric and/or oceanic CO₂ reservoirs which are possibly linked with the suggested worldwide Little Ice Age. This result suggests that the so-called 'pre-industrial' CO₂ level was not constant even over the few centuries preceding the important anthropogenic perturbation of the various CO₂ pools.

In the search for the atmospheric CO₂ level during pre-industrial times (before AD 1860, and on the scale of a few centuries), other kinds of records have been used, including the

chemical measurements of atmospheric CO₂ performed during the second part of the nineteenth century, the $\delta^{13}\text{C}$ record of tree rings, and the chemical analysis of sub-surface ocean waters. A recent critical review^{8,9} of the different types of record of the atmospheric CO₂ concentrations from pre-industrial times to 1958 concluded that CO₂ analysis of the ice cores currently seems to be the best, and most direct, method of determining the atmospheric CO₂ concentration before AD 1900. We can consequently consider that the D57 results presented here provide a most representative record of the atmospheric CO₂ background level for the 'pre-industrial' period.

Finally, the D57 record is also the first ice-core analysis to indicate clearly the increase in atmospheric CO₂ due to the burning of fossil fuels and probably also due to the anthropogenic influence on the biosphere over the nineteenth and twentieth centuries. Figure 2 shows this increase as recorded in the D57 core since AD ~1850, compared with other estimates discussed in refs 8 and 9.

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Structure of the ammonia synthesis catalyst

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The synthesis of ammonia from hydrogen and nitrogen over a reduced iron oxide catalyst is so well known^{1–5} and so widely used (current world capacity is close to 100×10^6 tons per year) that it may seem surprising that the mode of operation of the catalytic remains enigmatic. That both H₂ and N₂ must first dissociate at the catalyst surface is beyond dispute, but there is debate as to the role of various promoters (Al, K, Ca) which greatly improve the catalytic performance when added to the precursor Fe₃O₄. Although it is widely accepted that regions of paracrystallinity exist within the catalyst, no one has questioned its overall crystallinity. In this work, which entails *in situ* X-ray diffractometry, we provide evidence that the active catalyst contains substantial amounts of a non-crystalline phase. Without promoter, reduction of Fe₃O₄ in the same conditions yields only crystalline α -iron.

Even though there have been more than 100,000 catalyst formulations for the synthesis of ammonia, the catalyst of choice still remains that selected for the original Haber–Bosch process,

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promoted iron. How do these promoters exert their influence? The likely role⁶ of the alkali is to facilitate the dissociative chemisorption of nitrogen, the rate-determining step. The present consensus view^{5,7} on the role of alumina is that it serves as a textural promoter by stabilizing the α -iron phase, and preventing it from sintering, after its formation by reduction in the reactant gas stream, from the fused oxide precursor. In particular, it has been argued⁸ on the basis of *ex situ* X-ray diffraction studies, that individual spinel units (FeAl_2O_4) are built into the active α -Fe catalyst and that these units cause internal strain giving rise to the formation of so-called paracrystals. This paracrystalline state is characterized by slightly broadened X-ray diffraction lines, a fact confirmed recently in another study⁹. Note, however, that these investigations were performed with reduced catalysts which had been re-oxidized before the measurements. Model studies with iron single crystal surfaces revealed the highest activity for the (111) surface plane¹⁰, and the highest rate of nitrogen chemisorption is exhibited by this plane¹¹. The presence of potassium, however, was found to eliminate the activity differences between various crystal planes (without affecting their structures)⁶. In view of these findings, discussions as to which crystallographic faces of iron are chiefly involved in ammonia synthesis at real (that is, promoted) catalyst become less cogent. This will be underlined by the present results which shed new light on the structure of the catalyst in working conditions. They also prompt us to re-examine the accepted picture that the function of the Al_2O_3 promoter is to stabilize the α -Fe phase in the synthesis catalyst. The evidence suggests that the active state of the promoted iron catalyst could be non-crystalline. The catalyst possesses essentially no long-range atomic order in reaction conditions. Our results also show that a typical promoted catalyst precursor contains at least five distinct crystalline phases before reduction.

The experimental evidence has been acquired using, principally, *in situ* X-ray powder diffractometry, rendered possible by adapting a commercial cell¹², capable of repeated rapid and slow scans of 2θ (scattering angle) operating at temperatures of up to $\sim 550^\circ\text{C}$ and pressures of up to 1 bar. This set-up, which readily permits data accumulation, is a more sensitive apparatus than earlier versions¹³ and is capable of operation at temperatures higher than those explored, also *in situ*, by Mosesman¹⁴. Other measurements involving Mössbauer spectroscopy, physical adsorption, kinetic studies of ammonia formation rates at high pressure as well as a range of electron microscopic techniques all point to the catalytically active phase of iron being amorphous, in the sense that sput-cooled metals and alloys are amorphous. Full details of our work will be presented elsewhere. Here we concentrate on the results of the X-ray studies that have been carried out on typical industrial ammonia synthesis catalysts (provided by BASF (catalyst number MUC) and ICI).

The X-ray diffractogram for typical promoted BASF and ICI unreduced catalysts containing 2% Al_2O_3 , 2% CaO and 0.5% K_2O shows predominantly hkl reflections for Fe_3O_4 . However, a diffractogram which took ~ 100 h of recording time and involved three separate specimens with overlapped regions of 2θ (owing to suspected X-ray-induced radiation damage) shows the presence of a large number of weak diffraction peaks arising from minority phases. Analysis of such multiphase mixtures is not straightforward, and an unambiguous assignment using solely the diffractograms and the Joint Committee on Powder Diffraction Studies data file is not possible. When combined with knowledge of the known chemistry of Fe/Al and Ca/Fe oxides and previous information concerning the presence of CaO in promoted catalysts, the minority phases so identified were: FeAl_2O_4 , FeAlO_3 , CaO and $\text{K}_2\text{Fe}_{22}\text{O}_{34}$. Alternative assignments are possible, and could, for example, include CaFe_3O_5 and KHCO_3 , but the presence of the bicarbonate can be safely discounted since there is no (analytically measurable) carbon present in the catalyst.

Unpromoted catalysts yielded diffractograms containing reflections from Fe_3O_4 only. When these unpromoted catalysts

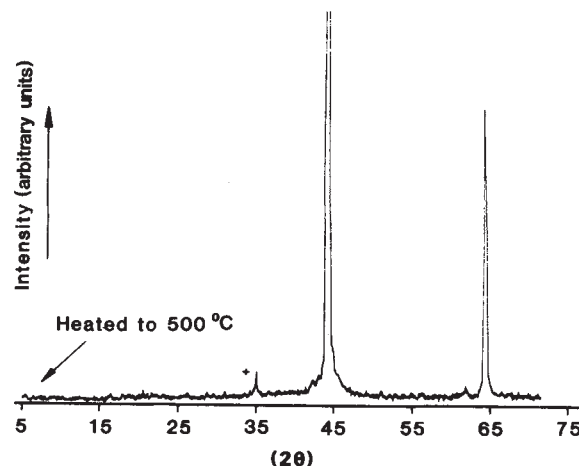


Fig. 1 Diffractogram (recorded at 500°C) of unpromoted magnetite catalyst that had been heated in 1 bar H_2 up to 500°C . The main peaks are attributable to α -iron. (The peak marked + is a spurious reflection from the high-temperature attachment.)

were heated in 1 bar H_2 ($30\text{ cm}^3\text{ min}^{-1}$) up to 500°C , the resulting diffractogram, recorded at this temperature with the catalyst still exposed to the H_2 atmosphere, revealed (Fig. 1) peaks attributable to α -Fe.

When the diffractograms were recorded with the promoted catalysts exposed to N_2 at a pressure of 1 bar and at temperature up to 500°C , they were indistinguishable from the patterns of the starting material. Dramatic changes in the diffractograms were, however, seen when the promoted catalysts were heated in either 1 bar of H_2 or 1 bar of $3\text{H}_2:1\text{N}_2$ mixture (Fig. 2). At $\sim 425^\circ\text{C}$ for the BASF catalyst and at $\sim 460^\circ\text{C}$ for the ICI catalyst, very broad diffraction peaks (~ 10 times as wide as those previously reported^{8,9} for paracrystalline iron) were obtained.

These diffraction patterns are very different from those expected for finely divided or paracrystalline α -iron. No intense reflections are observed, but instead there are a series of weak and broad features. The most surprising feature is a broad diffraction peak at $2\theta \sim 25^\circ$. There are no allowed diffraction lines for αFe at this angle and hence it cannot be interpreted in terms of extremely finely divided crystalline iron. Experiments monitoring the weight loss during reduction confirm that the material is essentially fully reduced ($\pm 1\%$) during our experiments and so the features displayed must be caused by a phase

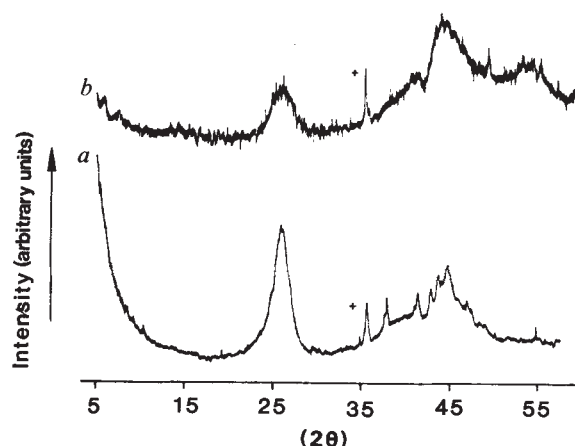


Fig. 2 Typical diffractograms of reduced promoted magnetite catalysts. *a*, Heated to 500°C ; *b*, subsequently cooled to 400°C . These broad diffraction peaks are much wider than any previously reported (see text). A new rather broad peak (at $2\theta \sim 25^\circ$) also appears irrespective of whether the reduction is carried out, at 1 bar, in either H_2 or $3\text{H}_2:1\text{N}_2$. The sharp peaks superimposed on the broad background arise chiefly from small particles of crystalline FeAlO_3 . (The peak marked + is a spurious reflection from the high-temperature attachment.)

which contains predominantly iron. The shape of the peak at 2θ 25° seems to depend on the conditions used during reduction (the temperature and gas flow), but we will not consider this in detail here because the effects may be obscured by the deviations from para-focusing caused by the sample expansion, and by the tendency for the sample to fall off. The diffraction pattern shows a striking similarity to that of some glassy metal alloys such as Fe/Ni/B which display a broad diffraction feature at $2\theta \sim 24^\circ$ when doped with a small quantity of silicon¹⁵. The simplest explanation of the data observed here is the formation of an amorphous iron-containing phase which is stabilized by the incorporation of small quantities of dopants that may arise from the promoter additives or from residual oxygen.

It is also possible to propose more complex assignments for the data, based on the fact that the broad feature at $2\theta \sim 45^\circ$ arise from allowed diffraction peaks of α -Fe. Thus, microcrystalline particles of α -Fe (~ 20 Å diameter) may be present together with an additional new phase of iron giving rise solely to the peak at 2θ 25°. Evidence for this view is less compelling than the simpler explanation that the material is amorphous. Whilst the diffraction pattern is dominated by the 'amorphous' features, the presence of some residual crystalline phases is indicated by small but comparatively sharp peaks in the region of 2θ from 35° to 55°. These have been identified as arising for the most part from FeAlO₃. Through the viewing port of the X-ray camera, the promoted catalysts were seen to expand rapidly during the reduction in H₂ at elevated temperature, a fluffy black powder resulting after the expulsion of water. These changes occurred concomitantly with the changes in crystallinity, detected by the repeat-scan X-ray diffractometry.

The diffractogram in Fig. 2a was recorded over an 18-h period with the sample exposed to 1 bar H₂ at 500 °C. The essential features of this diffractogram are obtained with the catalyst exposed to 3H₂:N₂ mixtures at elevated temperature (~ 425 °C) and are retained when the catalyst is cooled to room temperature in a non-oxidizing (H₂ or H₂-N₂ mixture) atmosphere. The catalyst spontaneously oxidizes on exposure to air even at room temperature and forms Fe₃O₄ indistinguishable from the non-reduced precursor. Other measurements (and all handling) were, therefore, carried out under a nitrogen atmosphere.

That this non-crystalline, reduced iron can be a catalytically active phase was confirmed by kinetic measurements (3H₂:1N₂ at total pressure of 9 bar in the temperature range 350–450 °C). At 400 °C, conversion rates were ~ 10 μmol of NH₃ per g of catalyst per s. This amorphous catalyst, nevertheless, retained some morphological texture, possibly because the coexisting microcrystalline FeAlO₃ there consist of numerous platelets (~ 200 Å dimensions); and, by transmission electron microscopy, we observed some mesopore structure. This is reminiscent of the small particles (~ 300 Å diameter) seen¹⁶ by scanning electron microscopy. No Mössbauer signal could be obtained^{17,18} in conditions which yielded clear signals from other iron-containing catalysts: this is what one expects from a catalyst that is more similar to a glass than a solid.

Our *in situ* X-ray diffractometric studies show that a promoted ammonia synthesis catalyst produced by reduction in hydrogen is largely non-crystalline interspersed with microcrystals of FeAlO₃ and other promoter phases.

Whilst more work remains to be done to elucidate the nature of the non-crystallinity, the precise effects of water vapour and the influence of FeAlO₃ during the reduction^{7,9}, it is clear that the degree of coordination of a surface Fe atom in the catalyst, and the apparently central role assigned to (111) faces, needs to be reconsidered.

If the main phase in the active promoted catalyst is not pure elemental iron and impurities such as promoter additives or residual oxygen in a total concentration of $\sim 1\%$ permit the formation of a disordered phase distinctly different from a material containing small particles of crystalline α -Fe, many of the numerous other catalyst formulations previously discarded could, in appropriate conditions of preparation, turn out to be very efficient.

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Liquid-phase polymer-based retention—the separation of metals by ultrafiltration on polychelatogens

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The importance of trace-element analysis in geological, biological, environmental and industrial processes has been increasingly recognized. Although modern instrumental methods of analysis have been applied, preliminary enrichment techniques are required to separate the components to be determined from the interfering constituents of the sample^{1,2}. For the separation of inorganic ions contained in natural waters, industrial fluids or dissolved solid materials, solvent extraction, sorption, ion exchange, precipitation and other methods have been used. However, techniques based on a two-phase distribution or on heterogeneous reactions often cause problems because, in contrast with homogeneous reactions, diffusion controlled processes must be used. Until now, no simple, rapid method has been available for the preconcentration and separation of metals in the homogeneous aqueous phase. Here we demonstrate a technique for the enrichment and separation of metals in aqueous solution using soluble non-crosslinked polymers with chelating groups (polychelatogens)^{3,4} called liquid-phase polymer-based retention (LPR).

A variety of water-soluble polymeric reagents with strong complexing properties have been synthesized and used, in combination with membrane filtration, to separate complex from non-complex metal ions in the homogeneous phase. The LPR method is based on the retention of metal ions by a membrane which separates macromolecules from low relative molecular mass compounds as the complexes form with the polymer from a sample solution.

The experimental arrangement is outlined in Fig. 1. The system consists of the membrane filtration cell, containing the polychelatogen solution, to which the sample is added. For separation and enrichment, the washing solution is passed to the cell from reservoir I under stirring. The filtrate contains all the metal ions which are not retained as macromolecular complexes by the polymer reagent. Large volumes of very diluted samples are first enriched in respect to metals of interest by passing the solution from reservoir II to the much smaller volume of cell solution. Thereafter, the additional separation process is carried out by adding washing fluid from reservoir I to remove non-bound metals from the cell.

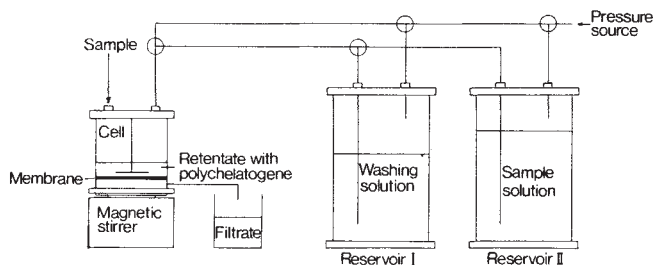


Fig. 1 The principle of metal separation with polychelators (from Chemische Fabrik Heyl) using membrane filtration. The sample solution is placed into the cell which contains an aqueous solution of polychelator (polymer reagent with chelating or other complexing groups). By adding the washing solution from reservoir I separation of metals is achieved by the selective retention of some of them in the retentate and the washing out of the others in the filtrate. Preconcentration of metals can be performed by passing the sample solution from reservoir II to the cell. The cell solution is then washed with the liquid from reservoir I.

The ultrafiltration cell is fitted with membranes, which retain molecules of molecular mass fractions greater than 10,000 (Berghof BM 10 or Amicon PM 10). Poly(ethyleneimine), poly(ethyleneimine-diacetic acid), poly(vinylpyrrolidone-co-vinylaminobutyric acid), poly(ethyleneimine-methylthiourea), and poly(acrylic acid-co-allylthiourea) act as polychelators in the cell as 1% aqueous solutions³. The average molecular mass of the polymer reagents is in the range 30,000–400,000 g mol⁻¹ (ref. 3). Capacity studies of the polychelators have been reported previously³. The unfunctionalized poly(ethyleneimine), for example, binds 0.6–2.4 mmol metal per g polymer depending on the pH.

The initial concentration of metals in the cell solution (40 ml) or in reservoir II varies from 0.1 to 10 p.p.m.; the pH of the cell and reservoir solutions are adjusted to the same value. The system is pressurized (300 kPa), the cell solution stirred for 5 min and then washed with the reservoir fluid at a flow rate of 4–6 ml min⁻¹. The metal contents of filtrate fractions and cell solution are analysed by atomic absorption spectrometry. Other analytical methods in solution can be used, in principle, for metal analysis of the retentate; electroanalysis is also applicable⁴.

The filtrate analysis data are used as the basis of the elution diagrams (Fig. 2) which show the metal concentration in the filtrate as a function of filtrate volume. If no metal can be detected in any filtrate fraction, complete retention has been achieved. When there is no retention, elution curves are obtained which can be approximated by the dotted curve in Fig. 2b. That curve is described by the exponential function:

$$C_f = C_0 e^{-V_f/V_0}$$

where C_f denotes the concentration of metal in the filtrate, C_0 the initial metal concentration in the cell, V_f and V_0 are the volumes of filtrate.

We have investigated the retention and separation of several metals using different polychelators. Typical results are shown in Table 1 for Co, Ni, Zn, Cd and Cu. Metal retention showed

Table 1 Retention of metal ions by poly(ethyleneimine) in the homogeneous phase at different pH values

pH	Retention (%)						
	Co ²⁺	Ni ²⁺	Zn ²⁺	Cd ²⁺	Cu ²⁺	Mg ²⁺	Na ⁺
2.1	1.2	<0.1	1.0	<0.1	86.3	—	—
3.2	16.3	21.3	0.5	0.7	>99.0	—	—
4.0	93.1	>99.0	—	98.1	>99.0	—	—
5.3	>99.0	>99.0	>99.0	>99.0	>99.0	<0.1	<0.1

Polymer concentration, 1%; initial metal concentration, 10 p.p.m.; filtrate volume was equal to 10 volumes of cell solution.

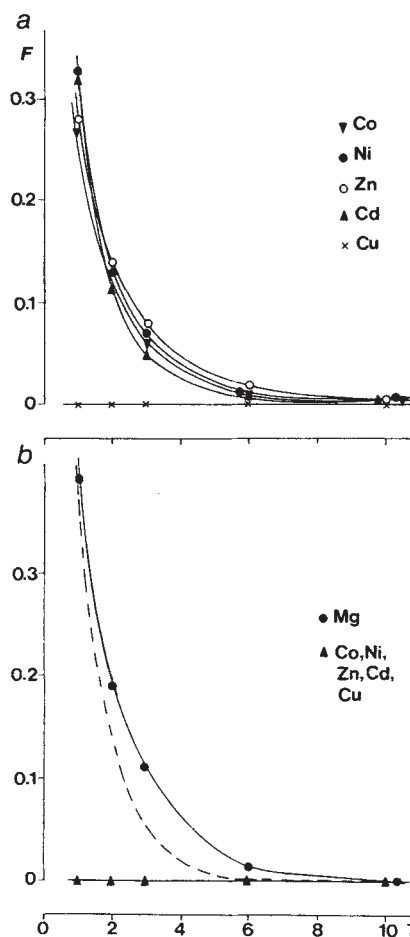


Fig. 2 Elution diagrams for some divalent metals separated by poly(ethyleneimine) using membrane filtration. Filtration quotient $F = C_f C_0^{-1}$ is plotted against the ratio $Z = V_f V_0^{-1}$ at pH 3.2 (a) and 5.3 (b); C_f and V_f , V_0 are concentration and volume of filtrate and cell respectively. Polymer concentration, 1%, $C_0 = 10$ p.p.m. Dotted curve, elution of an arbitrary metal which is not retained in the cell.

a strong dependence on pH, experiments were, therefore, performed at different pH values in the range 2–6. For example, >80% Cu was retained at pH 2.1 with $V_f = 10 V_0$ (Table 1). Complete retention of Cu was achieved at pH 3.2, whereas the other four metals were washed out in the filtrate (Fig. 2a, Table 1).

The elution profiles of the metals Ni, Zn, Cd and Co are similar to the theoretical curve in Fig. 2b. At higher pH values, these metals are retained completely by poly(ethyleneimine); thus, they can be separated from other elements not bound to the polymer. For example, Mg and Na ions, which do not form stable complexes with this polymer, are washed out even at pH 5.3 (Table 1).

Other polychelators with different functional groups have proved to be effective for metal retention using membrane filtration. Poly(ethyleneimine-diacetic acid) can retain 90% Cu at pH 2.2 and >99% at pH 5. At this pH value, Cu is also complexed effectively by the *N*-methylthiourea derivative of poly(ethyleneimine). Moreover, this reagent allows the quantitative retention of Cd and Hg. Using the copolymer of acrylic acid and *N*-allylthiourea, Hg is even retained at pH 1.9. Poly(vinylpyrrolidone-co-vinylaminobutyric acid) showed a lower retention tendency than do the other polychelators studied.

To apply this procedure to the analysis of very diluted samples it is important to be able to preconcentrate the elements to be determined. The LCP technique allows preconcentration by using two reservoirs as depicted in Fig. 1. To demonstrate this, a model fluid containing metal ions and 3% of NaCl (concentration in seawater) was passed from reservoir II to the cell. The

volume of the sample fluid was 10 times higher than that of the cell solution.

After 10-fold preconcentration, the cell solution was washed with distilled water from reservoir I. At a 10-fold volume of filtrate referred to the cell fluid >98% of Hg, Cd, and Cu were retained by poly(ethyleneimine-methylthiourea), whereas poly(ethyleneimine) retained 75–95% of Cu, Ni, Zn, Cd and Cu. Note that the concentration of Na in the cell was as low as 3–5 p.p.m. after the washing. This means that the Na concentration decreased by 10^4 times and that a factor of 10^5 (relative preconcentration factor) for the metals retained is achieved when taking into account the first preconcentration step (absolute preconcentration). Another absolute preconcentration step (up to one order) can be applied by reduction of the cell solution volume using conventional ultrafiltration. In addition, it is an advantage for the analysis that the sample can be handled in the homogeneous phase.

In conclusion, these polymeric reagents in combination with membrane filtration allow the enrichment and separation of trace elements in the presence of comparable and large excess quantities of sample constituents which could interfere with subsequent analysis.

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Evidence for an increase in cosmogenic ^{10}Be during a geomagnetic reversal

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Reversals in the geomagnetic field, which occur every few hundred thousand years, represent a dramatic change in the Earth's environment. Although there is no satisfactory theory for such reversals, it is generally accepted that the dipole field intensity decreases to <20% of its 'normal' value for a few thousand years during the change in direction¹. Because the galactic and solar cosmic rays which impinge on the Earth's atmosphere are charged, a significant fraction (about half) of them are deflected by the geomagnetic field². At the time of a reversal, this magnetic shielding is greatly reduced, and it has been suggested that the increased flux of high-energy particles could have effects on evolutionary³ or climatic⁴ processes. For example, the statistically significant coincidence in levels of some marine faunal extinctions and reversal boundaries in ocean sediments⁵ could be caused, directly or indirectly, by the decreased geomagnetic intensity during the reversal. We report here evidence in marine sediments for an increase in cosmogenic ^{10}Be production in the Earth's atmosphere during the Brunhes–Matuyama reversal 730,000 yr ago. In addition to confirming an increase in cosmogenic isotope production, the results provide information on the magnitude and duration of the geomagnetic intensity decrease during such an event, and the depth at which remanent magnetism is acquired in marine sediments.

In previous work, we found no significant evidence for increased ^{10}Be (half-life 1.5 Myr) at a reversal boundary⁶. Among the explanations we gave for this negative result was that the

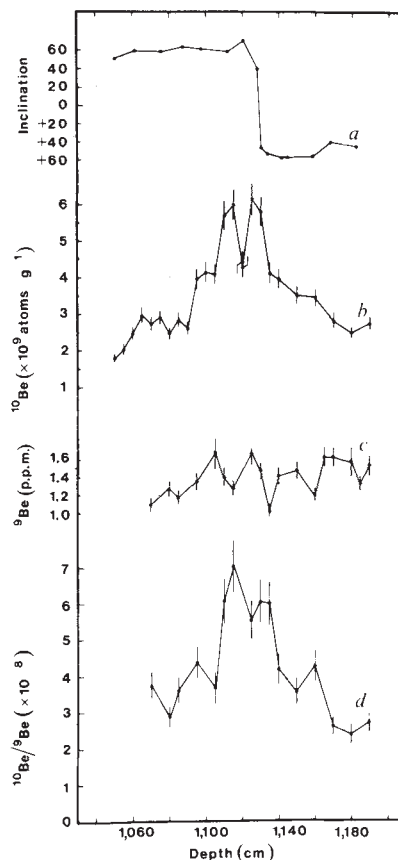


Fig. 1 a, Magnetic inclination; b, ^{10}Be concentration; c, ^9Be concentration; d, $^{10}\text{Be}/^9\text{Be}$ ratio as a function of depth in core V16-58.

sedimentation rate of the core studied (1.13 cm kyr^{-1}) was not high enough to retain a significant signal. We have thus looked at a core having a sedimentation rate of $\sim 2.5 \text{ cm kyr}^{-1}$. This core, V16-58, was taken at $46^\circ 30' \text{ S}$, $31^\circ 16' \text{ E}$ at a depth of 4,731 m. The material near the reversal zone is mostly diatomaceous lutite with thin interspersed layers of calcareous ooze⁷. The identification of the reversal level at $\sim 1,129 \text{ cm}$ as the Brunhes–Matuyama boundary, and the estimation of the sedimentation rate at this level are based on the biostratigraphy of Hays⁷, with more recent studies putting the 425,000-yr extinction level of the radiolarian *Stylatractus universus*⁸ at 420 cm (J. Morley, personal communication). The top part of the core has apparently suffered from a reduced sedimentation rate, hiatus or erosion.

Initial magnetic measurements, indicating a reversal level at $\sim 1,129 \text{ cm}$, as shown in Fig. 1, were carried out using procedures described in ref. 9. After the ^{10}Be results became available, the core was resampled and the position of the reversal confirmed. A more detailed palaeomagnetic investigation around the reversal level is now in progress.

For the ^{10}Be measurements, samples of 0.3–1 g were dissolved in the presence of 0.5 mg of ^9Be carrier, and chemical purification carried out as described earlier⁶. The ^{10}Be measurements were made on a Tandemron accelerator mass spectrometer facility, using the procedure described in ref. 10. Several thousand ^{10}Be events were recorded for each sample, and the estimated uncertainties are due to instrumental variations, as deduced from replicate measurements of standards¹⁰. The results are shown in Fig. 1. The low value at 1,120 cm during the initial series of measurements was so surprising that we processed and measured a second portion of the same sample. The good agreement between the two results both confirms the low value for this sample, and gives us added confidence that our assigned uncertainties are realistic.

As can be seen in Fig. 1, the ^{10}Be concentration increases by a factor of ~ 2 for samples near the reversal level. Before

ascribing this to a production increase, it is useful to consider other possible factors which could effect a change in ^{10}Be concentrations. For example, a change in the biological productivity, possibly associated with the reversal, could give rise to a variable biogenic component (opal or calcium carbonate) in the sediments; this would probably give a variable dilution of the ^{10}Be . Another possibility is that some change in environmental conditions might lead to an 'enrichment' of beryllium compared with other components in the sediment. To examine these alternatives, we have measured the stable isotope ^9Be over the same interval as the ^{10}Be . The ^9Be was measured by flameless atomic absorption spectrometry. Uncertainties in the ^9Be concentrations are estimated from the reproducibility of the measurements (in all cases except one, the results are the average of at least two determinations). The decision to measure ^9Be was made after the completion of the ^{10}Be measurements. Unfortunately, this meant we did not have material remaining for some of the levels, including the critical minimum at 1,120 cm, in which ^{10}Be had been determined.

Although there are variations in the measured ^9Be , these do not parallel those of ^{10}Be . In fact, the $^{10}\text{Be}/^9\text{Be}$ ratio, also given in Fig. 1, shows a peak similar to ^{10}Be alone. Unfortunately, this normalization may not be completely satisfactory. Although the oceanic geochemistry of ^9Be is still poorly known, we believe^{11,12}, contrary to Measures and Edmond^{13,14}, that a significant fraction of ^9Be may be deposited in marine sediments without coming to equilibrium with ^{10}Be . In such a case, the ^9Be would be an imperfect tracer for ^{10}Be , although it still should compensate for a variable biogenic component.

While the $^{10}\text{Be}/^9\text{Be}$ evidence strongly supports the interpretation of the observed increase of ^{10}Be concentration as being due to increased production, it is not necessarily a better monitor of ^{10}Be production variations. However, because of the similarity in the two curves (except for the minimum at 1,120 cm), the conclusions that follow are relatively insensitive to which one is used.

We also note that both ^{10}Be concentration and $^{10}\text{Be}/^9\text{Be}$ are of potential interest as dating tools^{11,15}. The strong variation in these parameters observed here demonstrates that caution must be exercised when dating by such procedures near reversal levels. In a preliminary study, Ku *et al.*¹⁵ have also observed a high ^{10}Be concentration in a single sample near the Brunhes-Matuyama reversal level. However, they also found an abnormally low concentration in a sample nearby, so the origin of their variations is not clear.

The ^{10}Be (or $^{10}\text{Be}/^9\text{Be}$) signal is not expected to reflect directly the geomagnetic palaeointensity profile for two reasons. First, the effect of the magnetic field on production rate is a function of geomagnetic latitude². The degree to which mixing in the atmosphere and the ocean average out this variation is not yet established. However, the mid-latitude location of V16-58, in a region of significant input of stratospheric aerosols, means that its sediment probably should reflect a reasonably 'average' production effect. Second, once the ^{10}Be is incorporated in the sediment, it is subject to mixing processes by benthic organisms. To illustrate the potential effect of this bioturbation, we adopt a model¹⁶ in which the sediment is assumed to be completely homogenized over a given depth, called the mixed layer, with no mixing below this depth. A more sophisticated model has been proposed which treats the mixing process explicitly by diffusion¹⁷. However, we do not have independent information on the appropriate diffusion constant for this core. For diffusion constants $>100\text{ cm}^2\text{ kyr}^{-1}$ the uniform mixing assumption is a good approximation to the diffusion model.

Adopting a mixed-layer thickness of 10 cm, we calculated output profiles for several different assumed input profiles. Figure 2 shows an example of one of these which fits the observed ^{10}Be profile reasonably well. As can be seen in Fig. 2, the bioturbation has three effects. It attenuates the maximum increase in production rate; it tends to smooth out rapid variations; and it moves the ^{10}Be levels down by approximately the assumed mixing depth.

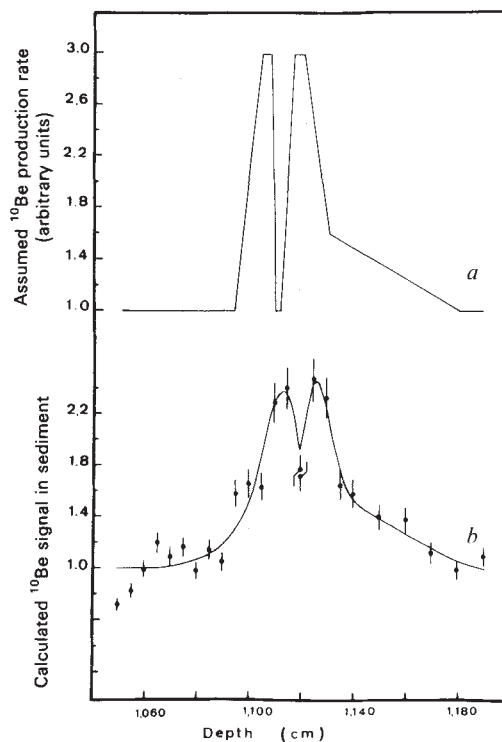


Fig. 2 a, Assumed ^{10}Be production rate profile; b, solid curve is calculated signal in sediment, using the bioturbation model described in the text, with a mixing depth of 10 cm. Points are experimental data from Fig. 1.

The input profile of Fig. 2 is, of course, not unique, and others which fit the experimental data equally well, or better, could be found. However, we do have some constraints. First, the maximum increase in production during the reversal cannot be significantly larger than the factor of 3 we have chosen, which corresponds essentially to a zero-intensity magnetic field². Second, the total area under the input and observed curves must be the same. Thus, the input function in Fig. 2 should represent at least the major features of the actual production curve. In particular, the mean duration for the production increase (and thus magnetic intensity decrease) should be approximately correct. As can be seen in Fig. 1, the interval of increased ^{10}Be is significantly longer than the time taken for the reversal of inclination. Such a result is consistent with other evidence which suggests that the geomagnetic field intensity is low before and after the change in inclination^{1,18}. From Fig. 1, the interval of increased production is $\sim 30\text{--}60\text{ cm}$, depending on what level of increase is chosen. Assuming a sedimentation rate of 2.5 cm kyr^{-1} , this interval corresponds to $\sim 12,000\text{--}24,000\text{ yr}$; this is similar to the time interval for the decreased magnetic field at another reversal, as estimated in ref. 18.

As can be seen in Fig. 2, the minimum at 1,120 cm implies a very significant decrease in production. However, the magnitude and duration of this decrease are obviously very strongly dependent on the parameters of the bioturbation model used. Thus, even if this decrease is due to an increased field intensity, the quantitative nature of the required change remains uncertain. It is interesting, however, that other authors have also found evidence for high intensities midway through reversals^{19,20}. More definite conclusions along these lines will necessitate more detailed ^{10}Be and palaeomagnetic profiles, comparison between several cores and more reliable bioturbation modelling. With regard to the latter, although the bioturbation depth used here is fairly typical^{16,17}, we wonder whether it can be reconciled with the rather abrupt variations seen in ^{10}Be , ^9Be and calcium carbonate⁷, over intervals as small as 5 cm. Piseras has recently made the same point regarding oxygen-isotope profiles in other cores²¹. Reduced bioturbation would mean that the actual ^{10}Be

production profile would approach more closely the observed ^{10}Be profile.

As we have discussed earlier⁶, the location of the ^{10}Be peak compared with the reversal level can also provide information on the depth at which remanent magnetization is acquired in marine cores. In the present case, it can be observed that the midpoint in the ^{10}Be peak is ~ 10 cm above the change in magnetic inclination (or ~ 15 cm if we use the input profile of Fig. 2). Neglecting the residence time of ^{10}Be in the ocean²², and assuming that the ^{10}Be midpoint corresponds to the change in direction, this would indicate that the magnetic remanence was 'locked in' at a depth of ~ 10 – 15 cm in the sediment (or slightly more, allowing for later compaction). This is consistent with the idea that the magnetic remanence is acquired at the base of the bioturbation zone.

We have presented the first direct evidence for increased cosmic ray radiation in the Earth's atmosphere during a geomagnetic reversal. Making some reasonable assumptions, the data also allow one to deduce significant information on the magnitude and duration of the magnetic intensity decrease during a reversal, and the depth at which detrital magnetic remanence is acquired in a marine sediment. It will be interesting to pursue this type of study at other reversal boundaries to determine whether there are significant differences in their intensity profiles, and, if so, whether they could be correlated with other features such as the severity of faunal extinctions. This work also demonstrates that ^{10}Be should be a potentially powerful

tool for testing the global nature of various geomagnetic 'excursions' that have been observed during the Brunhes epoch²³.

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Re-dating the English art-historical tree-ring chronologies

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Precisely dated oak (*Quercus petraea* Liebl. and *Q. robur* L. and their hybrids) tree-ring chronologies that show consistent cross-dating from Germany to England to Ireland^{1,2} have been labelled Type H³. In contrast, there have been difficulties in specifying the exact date ranges of some oak chronologies (termed Type A)³ constructed from art-historical timbers in England^{4,5} and the Netherlands^{6,7}. It has been asserted that the oak planks and boards used in the English art-historical chronologies derived from England and Flanders^{3,8}. The failure of these chronologies to date uniquely against Type H chronologies from the British Isles has led to suggestions that the timbers were imports, probably from the Baltic area^{1,9}, views supported by historical evidence^{10,11}. It has also been suggested that the dating procedure used for these chronologies has an element of circularity which could result in an erroneous placement of the chronologies going undetected¹². Evidence is presented here which indicates that the art-historical chronologies have been incorrectly dated, that they represent imports into England and Flanders and that failure to take account of exotic origins has led to the use of unsuitable estimates for missing sapwood.

In 1975, information on cold winters in the fifteenth century was derived from a section of art-historical oak tree-ring chronology, MC18, dated¹³ to AD 1234–1550. This section of chronology had originally been designated⁴ to the years 1230–1546 and subsequently reference chronologies related to MC18 (designated Refs1–4) were returned to this same '1546' dating^{3,5,14}. These chronologies, all >270 yr in length, form an internally consistent group covering the period from the early

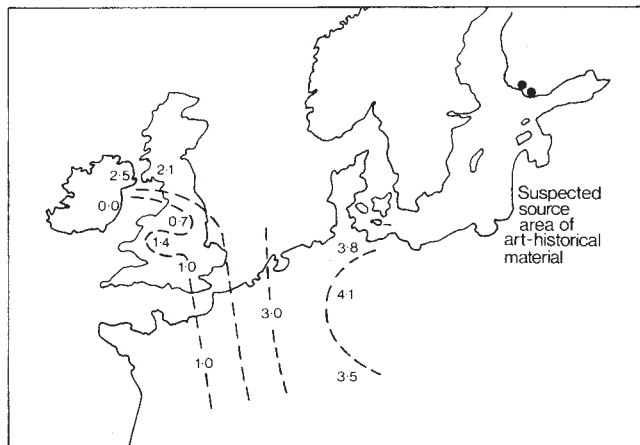


Fig. 1 Mean 't' values for the nine art-historical chronologies compared with 11 available Type H chronologies as in Table 1c, with the Type A chronologies in the 4-yr-forward or '1550' position.

●, The source of the Finnish oak sapwood values.

twelfth to the early seventeenth centuries. At other times Refs1–4 have been moved 1-yr-back, equivalent to a '1545' dating^{8,15,16} of MC18. The possibility of a circular argument in the dating of these art-historical chronologies has recently been discussed in an article which also gives documentary evidence for importation of oak planks and boards into England and Flanders from the Baltic during the later Middle Ages¹². Since that publication, three new pieces of evidence relevant to these Type A chronologies have become available.

First, one of us (J.H.) acquired some medieval timbers from excavations at Ipswich. The mean ring pattern of three timbers was found to span the years AD 1128–1293 by cross-dating against the established, and absolutely dated, German Type H (after Huber²) chronologies of Eckstein (personal communication) and Delorme¹⁷. The correlation values between the Ipswich mean ring-pattern and these two chronologies, obtained using the Belfast CROS program¹⁸, were 't' = 7.2 and 't' = 4.1 respectively for overlaps of 166 yr (normally 't' values >3.5 in association with overlaps of more than 100 yr are regarded as significant indicators of a likely tree-ring match). No significant

correlations could be found with any of the existing British Isles Type H oak chronologies. However, when this Ipswich ring-pattern was compared with the Ref4 Type A chronology⁵, a significant correlation value ($r = 5.3$, overlap 166 yr) was obtained with Ref4 ending not in AD 1399 as published, but in 1403, that is, equivalent to the 4-yr-forward or '1550' dating used in 1975¹³.

Almost immediately an exactly analogous situation was found by another one of us (M.B.). The mean ring pattern of two boards from a seventeenth-century painting of St Matthew was dated to the years AD 1326–1625 against the Type H German chronologies of Hollstein¹⁹ and Becker²⁰ with $r = 10.6$ and 11.3 for overlaps of 300 yr in each case. When compared with the published Type A chronologies, significant correlation values were obtained at the '1550' position ($r = 5.1$ for Ref3, $r = 4.6$ for Ref2 with overlaps of 270 and 246 yr respectively).

Following these results, we re-examined the dating of the Type A chronologies. Most workers outside the art-historical field had assumed that more persuasive evidence must exist for the '1545' and '1546' chronology placements than for the '1550' placement and that all of the possibilities would have been explored by the art-historical workers. Table 1 shows the correlations obtained for all of the available Type A chronologies, namely MC18⁴, Refs1–4⁵ and variants NewRef1, NewRef2/3 and Ref2/3/5 (J. M. Fletcher, personal communication), when compared with the published Type H chronologies from Göttingen¹⁷, Hamburg (ref. 21 and D. Eckstein, personal communication), South Germany²⁰, West Germany¹⁹, Belfast²², Scotland²³,

Giertz²⁴, northern France (J. R. Pilcher, personal communication), English MC10 (J. M. Fletcher, personal communication), Sheffield²⁵ and Dublin²⁶ at the '1545', '1546' and '1550' positions.

Consistent with the Type A dating suggested by the Ipswich and St Matthew examples above, there is strong correlation between the Type A chronologies and the principal published German Type H chronologies at the '1550' position. There is much less agreement with chronologies from the British Isles at this position. There is no consistent agreement with the Type A chronologies placed at the '1545' or '1546' positions. Figure 1 shows how, at the '1550' placement, the mean correlation values for the Type A chronologies increase as they are compared with more easterly chronologies. The values are not high enough to support an origin for the art-historical timbers within Germany (see, for example, the correlations obtained for the St Matthew boards) but they clearly indicate a likely origin to the east of the German chronologies. This would be consistent with an eastern Baltic origin which has been suggested on historical grounds¹².

But why has this level of consistent correlation (against published chronologies) which indicates a '1550' dating been ignored and why was this same dating, used¹³ in 1975, overridden? It is referred to as being "discarded on account of poor visual agreement"⁵ and elsewhere the temporary use of the '1550' position is accounted for by a "4-yr periodicity in our oak chronology"²⁷.

In a '1550' re-dating of the art-historical chronologies, the time left between the last heartwood rings of surviving ring

Table 1 Correlation values obtained using the Belfast CROS program¹⁸ for comparisons of nine art-historical chronology variants with precisely dated Type H chronologies

a 1-yr-back '1545' position

	MC18	Ref1	Ref2	Ref3	Ref4	Ref 1234	New Ref1	New Ref2/3	Ref 2/3/5	Mean
Göttingen	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.2
Hamburg	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
S. Germany	0.0	0.0	0.0	0.2	1.3	0.2	0.0	1.1	0.8	0.4
W. Germany	0.0	0.0	0.1	1.0	1.9	1.4	0.0	1.4	1.3	0.8
Belfast	1.0	2.7	1.5	0.0	0.5	1.4	1.8	1.8	2.4	1.5
Scotland	0.0	0.7	1.1	0.0	0.0	0.0	0.0	0.1	1.4	0.4
N. France	0.3	1.0	2.0	0.3	1.2*	1.4	0.1	1.6	1.6	1.1
England/Wales	0.4	1.0	3.3	1.7	†	2.1	0.7	2.4	2.2	1.7
Sheffield	0.0	0.6	1.5	0.9	†	1.1	0.0	1.1	0.3	0.7
MC10	0.0	0.6	1.5	2.8	†	1.9	0.2	2.1	1.2	1.3
Dublin	0.4	1.0	1.4	1.5	†	1.7	0.1	2.1	1.4	1.2

b '1546' position

Göttingen	0.5	0.4	0.1	0.0	2.2	1.7	1.4	0.0	0.7	0.8
Hamburg	1.0	1.7	1.0	0.0	2.9	2.5	2.0	0.1	1.1	1.4
S. Germany	2.1	1.8	1.3	0.8	3.8	3.2	2.5	0.0	1.3	1.9
W. Germany	0.7	1.0	1.4	0.0	2.3	1.5	1.4	0.0	0.6	1.0
Belfast	1.3	0.5	0.0	0.0	0.5	1.2	0.5	0.8	1.1	0.7
Scotland	0.1	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.1
N. France	2.2	1.7	0.3	0.0	1.1*	0.8	2.4	0.0	0.0	1.0
England/Wales	0.0	0.0	0.0	0.1	†	0.0	0.1	0.0	0.0	0.0
Sheffield	0.0	0.0	0.0	0.0	†	0.0	0.0	0.0	0.0	0.0
MC10	0.0	0.0	0.0	0.0	†	0.0	0.0	0.0	0.0	0.0
Dublin	0.0	0.0	0.0	0.0	†	0.0	0.0	0.0	0.0	0.0

c 4-yr-forward '1550' position

Göttingen	3.3	1.8	4.5	4.6	3.7	5.3	3.3	4.2	6.1	4.1
Hamburg	3.4	2.7	2.7	2.4	4.8	5.9	3.5	2.8	5.6	3.8
S. Germany	3.2	2.0	4.2	4.7	2.6	4.8	2.8	4.0	3.4	3.5
W. Germany	2.2	2.0	2.7	4.1	2.4	4.1	2.4	3.5	3.2	3.0
Belfast	3.0	1.5	1.1	3.0	1.3	3.2	1.3	4.6	3.3	2.5
Scotland	1.8	2.2	0.0	1.5	3.6	4.0	1.7	1.0	2.7	2.1
N. France	0.0	0.5	2.0	1.5	0.0*	1.6	0.1	1.0	2.0	1.0
England/Wales	1.2	2.0	0.3	2.3	†	2.2	1.5	1.1	1.9	1.4
Sheffield	0.7	1.8	0.3	0.7	†	1.0	0.7	0.6	0.1	0.7
MC10	1.3	1.6	0.4	0.4	†	1.0	0.8	0.8	1.6	1.0
Dublin	0.0	0.3	0.0	0.0	†	0.0	0.0	0.0	0.0	0.0

Ref*1234" is the mean of the published⁵ Refs1–4. 0.0 indicates zero or negative correlation.

* An overlap of <130 yr. † An overlap of <65 yr. All other overlaps are >160 yr. a, With the art-historical chronologies at the '1545' position, b at '1546' and c at '1550'.

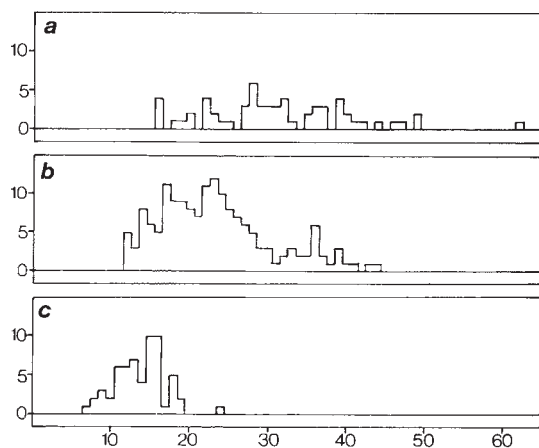


Fig. 2 Frequency of sapwood numbers in oaks from: a, England^{12,28}; b, West Germany²⁹; c, Finland.

patterns and the use dates for 'known age' paintings is shortened by 4 yr. In one of the examples used by Fletcher *et al.*⁴ (Thomas, second Baron Wentworth, painted 1568), the '1550' chronology placement leaves only 20 yr between its last heartwood ring and 1568. This period has to account for both sapwood and seasoning factors. In the case of the First-Viscount-Montague panel (painted 1569) only 15 yr are left to account for possible missing heartwood, missing sapwood and seasoning. Such intervals would be unacceptably short for English panels given the numbers of sapwood rings observed on modern English oaks^{12,28} and it may have been the erroneous belief in an English origin for the panels which made the possible '1550' dating unacceptable.

If our revised dating is accepted, it suggests that the number of oak sapwood rings in the area from which the panels derived must have been less than the numbers normally accepted in England. Sapwood on mature oaks tends to be shorter in Western Germany²⁹ and substantially shorter for Roman (mean 18, range 11–27) and Dark Age (mean 17, range 11–21) oaks in South Germany^{20,30}. We now have evidence for oaks from the eastern Baltic as the result of field work by members of the University of East Anglia Climatic Research Unit in 1984. The samples are 60 cores from 49 trees from two sites in southwestern Finland—Ruissalo and Solbole (all trees were *Q. robur*). The mean number of sapwood rings is 13.9 with a standard deviation of 3.2 and values range from 7 to 24. All trees were >120 yr in age. The mean number of sapwood rings from the Finnish sites is significantly smaller than the English sapwood values at the $P < 0.0001$ level (Fig. 2).

These results suggest that: (1) an eastern Baltic origin for the art-historical chronologies as suggested on historical grounds; (2) a movement of the art-historical chronologies forward to a '1550' placement; and (3) a very short sapwood interval are all compatible. When vindicated by subsequent work on Polish or Lithuanian timbers, these results make it inevitable that all of the art-historical datings derived using the '1545' or '1546' chronology placements, and the specifically tailored sapwood estimates favoured by some workers^{15,31}, will have to be revised. If the German art-historical dendrochronologists favour a '1548' chronology placement for their equivalent Netherlands II chronology³, then all their dates will move forward in time by 2 yr. However, their estimated felling dates may have to be additionally revised in the light of the very short Baltic sapwood observations above.

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Patterns of mortality and age at first reproduction in natural populations of mammals

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There is great variation in the age at which females of different mammalian species first breed. Recent comparative analyses have focused on the relationship between age at first reproduction and body size^{1–9}, but differences in patterns of mortality experienced by natural populations are expected to have major effects on selection for age at first reproduction^{10–14}. Here we show that the age at which females first reproduce is strongly correlated with expectation of life at birth, after the effects of body size have been removed, within and among species of mammals living in natural populations.

Some shrews give birth when they are only 7 weeks old¹⁵, whereas female African elephants produce their first offspring at an age of some 15 years¹⁶; such differences in age at first reproduction are highly correlated with the species' differences in body size^{1,2,8,9}, suggesting that the time required to grow to adult size determines the age at which mammals become sexually mature^{2,4}. Consistent with this idea, the timing of many physiological and reproductive processes (for example, cardiac cycle time and maximum recorded lifespan) scale in similar ways with body size, approximately to the 0.25 power^{4,8}.

However, body size might be a confounding factor in the sense that it influences one variable which in turn influences some other variable; both variables would then be correlated with body size. For example, species with high rates of mortality in the wild might be selected to mature earlier than those which are long-lived, but rates of mortality might be influenced by body size (larger species can defend themselves against predators more effectively and they can survive for longer periods without food⁶). Data are now available on mortality patterns,

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body sizes and age at first reproduction of females for 29 mammal species (representing 25 genera and 6 orders) from natural populations with stable age distributions¹⁷. The species range in weight from 20 g to 4,000 kg. In addition, equivalent data are available from six populations of a further species, the Columbian ground squirrel, *Spermophilus columbianus*^{18,19}. These data can be used to test the hypothesis that differences in patterns of mortality are correlated with the ages of the females at first reproduction.

Six of the species in the sample belonged to two genera (*Spermophilus* and *Peromyscus*) and, to reduce taxonomic bias, analyses were performed at the generic level (that is, on the averages of logarithmically transformed congeneric species' values). All variables were logarithmically transformed before analysis. As a summary measure of mortality, we have used Millar and Zammuto's¹⁷ estimates of mean life expectancy at birth in years (a comparison of the use of this versus other measures will be presented elsewhere). Genera with low mortality have a high expectation of life at birth. The correlation between age of females at first reproduction and life expectancy at birth is high and positive ($r = 0.976$, $n = 25$, $P < 0.001$). This correlation, however, might result from independent correlations of each variable with body size, but this seems not to be the case since the two variables are more highly correlated with each other ($r = 0.976$) than either is with body size ($r = 0.894$ and 0.873 respectively, $n = 25$ in both cases), and they remain highly correlated after the effects of body size have been removed by partial correlation ($r = 0.892$, $n = 25$, $P < 0.001$). The latter relationship means that mammals with an unexpectedly long lifespan (relative to body size) start reproducing relatively late (again relative to body size). The relationship is demonstrated graphically in Fig. 1—note the taxonomic spread. Genera from each of the two best-represented orders, the Artiodactyla and the Rodentia, cover most of the ranges of relative age at first reproduction and relative life expectancy. The removal of size effects is also evident: values for squirrels lie near that for the elephant, and the value for mice near that for the warthog. A positive partial correlation between age at first reproduction and life expectancy at birth, with the effects of body size removed, also holds among populations of the Columbian ground squirrel ($r = 0.874$, $n = 6$, $P = 0.05$).

Probably as a consequence of constraints imposed by seasonality, seven of the genera in the cross-species sample mature as 1-yr-olds (ranging in weight from the 0.1-kg *Tamias* to the 7.5-kg *Lynx*). When the analysis is repeated without these genera, the correlation between age at first reproduction and life expectancy (with body size effects removed) remains high and significant ($r = 0.836$, $n = 17$, $P < 0.001$). Of course, differences in body size may still influence age at first reproduction. Elephants could not mature and produce offspring in the few weeks that it takes shrews or mice to mature and, indeed, the partial correlation between age at first reproduction and body size with the effects of life expectancy removed is positive, though barely significant ($r = 0.390$, $n = 25$, $P < 0.1$).

To test for the possibility that gestation length, litter size, neonatal weight or litter weight was ultimately responsible for the correlation between age at first reproduction and life expectancy, data on these variables for the species in the sample were extracted from the literature^{20–24}, and the effect of each new variable was removed by partial correlation. With the effect of each variable removed, in no instance did the correlation between age at first reproduction and life expectancy fall below 0.9.

The positive relationship between age at first reproduction and expectation of life at birth, after removal of the effects of body size, calls for an explanation in terms of the costs and benefits of reproduction at different ages. Perhaps the most straightforward interpretation is that each bout of reproduction reduces subsequent reproductive success (either by further increasing mortality or by decreasing fecundity), and that older mothers produce larger litters (and/or more viable offspring) than do younger mothers¹⁹. As life expectancy at birth decreases

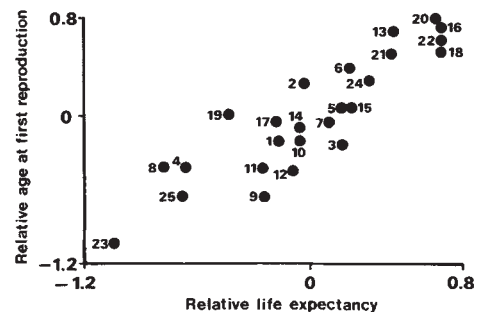


Fig. 1 Relative age at first reproduction plotted against relative life expectancy at birth for natural populations of mammals. Relative values refer to deviations from logarithmic regression lines of age at females' first breeding, or expectation of life at birth, on adult female body size. Numbers refer to different mammalian genera. Artiodactyla: 1, *Syncerus* (buffalo); 2, *Hippopotamus*; 3, *Aepyceros* (impala); 4, *Sus* (pig); 5, *Cervus* (deer); 6, *Ovis* (sheep); 7, *Hemitragus* (tahr); 8, *Phacochoerus* (warthog); 9, *Kobus* (kob); 10, *Connochaetes* (gnu). Carnivora: 11, *Taxidea* (American badger); 12, *Lynx*; 13, *Lutra* (river otter); 14, *Mephitis* (skunk). Lagomorpha: 15, *Sylvilagus* (cottontail rabbit); 16, *Ochotona* (pika). Perissodactyla: 17, *Equus* (zebra). Proboscidea: 18, *Loxodonta* (African elephant). Rodentia: 19, *Castor* (beaver); 20, *Tamias* (chipmunk); 21, *Spermophilus* (ground squirrel); 22, *Sciurus* (grey squirrel); 23, *Clethrionomys* (vole); 24, *Tamiasciurus* (red squirrel); 25, *Peromyscus* (mouse).

under such circumstances, selection will favour maturation at earlier ages. (This interpretation may also be consistent with an apparently similar relationship, pointed out by Lack²⁵, between age at first reproduction and patterns of mortality among families of birds.) At a more proximate level, nutrition levels may influence the correlations. High nutrition levels can result in early maturation^{26,27} and decreased lifespan²⁸ within and among mammal species, for example, Columbian ground squirrel populations mature earlier and have shorter life expectancies when natural food resources are more abundant^{18,19}.

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An antiemetic is antidotal to the satiety effects of cholecystokinin

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Although cholecystokinin (CCK) has been proposed as a satiety agent¹⁻⁴, this property has been disputed by some who claim that the compound exerts its 'satiety' effects by inducing aversion⁵⁻⁷. We considered that if CCK-induced reductions in food intake occur through the mechanism of normal satiety, CCK-induced satiety and normal satiety should respond in the same way to a pharmacological challenge. We demonstrate here that the administration of an antiemetic to rats significantly attenuates the food intake reduction caused by exogenously administered CCK but does not increase normal consumption. The effects of endogenous CCK are therefore quite different from those of exogenous CCK, making any previous study equating exogenous CCK effects with natural satiety problematic.

Ninety-eight male Wistar rats (300–450 g) were housed and tested in individual wire mesh cages and maintained on a 12-h light/dark cycle. Tap water was available *ad libitum* and a maintenance diet of Purina rat chow was available for 2 h each day, beginning at 17.00 h. The rats were trained to drink a 1:1 Mazola corn oil/water solution emulsified with 1% lecithin (a diet expected to stimulate endogenous CCK release^{8,9}) for 30 min each day after a 17-h period of food deprivation and were habituated to receive intraperitoneal (i.p.) injections of physiological saline. Rats were then assigned to one of four conditions matched by mean intake and standard deviation—saline-saline (S-S), antiemetic-saline (A-S), antiemetic-CCK (A-CCK) and saline-CCK (S-CCK). Each rat received two injections and was then given the emulsion to drink for the usual 30 min. The first drug for each group was given 30 min before the rat received his oil and the second drug immediately before oil presentation. The saline-treated rats received 0.9% saline solution (1 ml per kg, i.p.) and the antiemetic-treated rats received trimethobenzamide (5 mg ml⁻¹ per kg, i.p.). (This drug and dose was used by Coil *et al.*¹⁰ to show that antiemetics could be used to attenuate LiCl-induced aversions. Trimethobenzamide is an antiemetic which acts specifically on the chemoreceptor trigger zone and has been shown to be useful in alleviating nausea in humans.) Cholecystokinin octapeptide (Sincalide; Squibb) was administered i.p. at 5, 10, 20 or 40 Ivy dog units (IDU) per ml per kg.

The different CCK doses were tested in three successive sets of new naive rats, the initial oil and saline injection training being the same for each set. In the first set, after oil intake stabilization, 28 rats were divided equally into the four conditions, with the CCK rats receiving a dose of 20 IDU per ml per kg i.p.; in the second set, another 28 rats received CCK at 10 IDU per ml per kg, and in the final set, the extreme CCK doses (5 and 40 IDU) were given to one S-S group and one A-S group ($n = 42$). (Because the S-S and A-S controls were so stable in the previous two sets, only one S-S and one A-S control (seven rats in each) was run for each of the two CCK doses.)

One score was obtained for each rat by comparing the percentage consumption on the experimental day with the mean consumption for the previous 3 days. Per cent mean intake scores were used rather than absolute intake scores, to minimize any potential differences in average drinking between the three sets of this experiment. The day of the experimental manipulation and the group of animals chosen for each set had no significant effect on the results. For the two control conditions (S-S and A-S), there was no significant difference in intake from one run to the next. An analysis of variance showed that the per cent mean intake between sets was not significantly different ($F =$

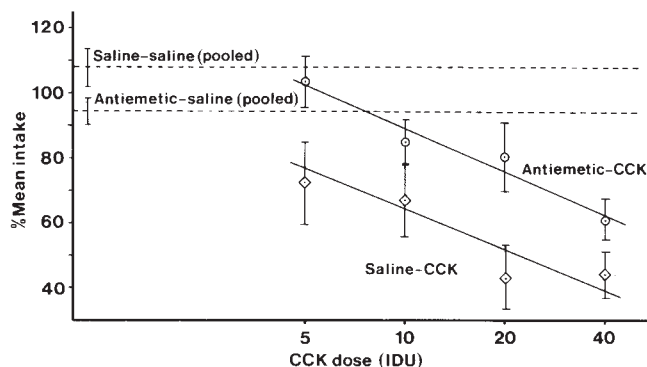


Fig. 1 Per cent mean intake (s.e.m.) of oil emulsion under four conditions of drug injections: saline-saline, antiemetic-saline, antiemetic-CCK and saline-CCK. The control S-S scores and the control A-S scores are pooled and are represented as dotted lines.

0.49, d.f. = 2, 36, $P > 0.05$). Furthermore, individual t -tests between an S-S and any other S-S condition and t -tests between an A-S and any other A-S condition showed no significant differences.

The pooled S-S group (from all three sets) consumed 108.1% of its normal amount on the experimental day (Fig. 1) and the pooled A-S group consumed 94.5% of its normal amount. Whereas neither the S-S group nor the A-S group showed significantly different consumption from normal (respectively $t = 1.30$, $n = 21$, $P > 0.05$ and $t = 1.30$, $n = 21$, $P > 0.05$), the A-S group showed marginally lower consumption than the S-S group ($F = 3.21$, d.f. = 1, 36, $P < 0.05$, one-tailed only). The antiemetic therefore did not increase normal consumption and, if anything, slightly reduced it.

The antiemetic attenuated the effect of CCK, results with the A-CCK group being significantly different from those of the S-CCK group ($F = 14.7$, d.f. = 1, 48, $P < 0.001$). The antiemetic (A-CCK) scores were higher than the saline (S-CCK) scores for all doses of CCK administered. Furthermore, whereas consumption in the S-CCK groups was always significantly different from their control (S-S) ($t = 4.90$, d.f. = 12, $P < 0.001$ for 40; $t = 4.01$, d.f. = 12, $P < 0.01$ for 20; $t = 2.84$, d.f. = 12, $P < 0.05$ for 10; $t = 2.59$, d.f. = 12, $P < 0.05$ for 5), that in the A-CCK groups was significantly different from their control (A-S) only for the highest CCK dose ($t = 2.67$, d.f. = 12, $P < 0.05$ for 40; $P > 0.05$ for 20, 10 and 5 IDU per ml per kg).

Because the antiemetic increased intake under CCK by about 26% for each dose of CCK used, if exogenous CCK were equivalent to natural satiety, normal intake with the antiemetic should have increased by 26% also. Instead, the antiemetic-treated rats consumed only 95% of their normal amount, rather than the expected 134%.

Clearly, exogenously administered CCK cannot be used as a model for natural satiety. Antiemetic administration increases eating after CCK injection, eliminating the supposed satiety effect of exogenous CCK. However, the antiemetic reduces or leaves unaffected normal eating when no CCK is given. The factors producing food intake reduction through CCK injection and the factors causing normal meal regulation therefore cannot be the same. Because the antiemetic is antidotal to CCK and because the antiemetic is not antidotal to normal satiety, it seems very likely that the factor causing food intake reductions after CCK administration is malaise and that malaise is not a factor producing normal satiety.

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Segregation of pathways leading from area V2 to areas V4 and V5 of macaque monkey visual cortex

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V5 and V4 are areas of macaque monkey prestriate visual cortex that are specialized for involvement in different aspects of visual perception, namely motion for V5 (refs 1–4) and colour vision, with other possible functions, for V4 (refs 2, 5–9). Thus, it is unlikely that they should be fed the same information for further processing, yet both receive a strong input from patches of the upper layers of V2 (refs 10, 11), the area immediately adjoining the primary visual cortex, V1. V2, however, seems to comprise functionally distinct subregions, which can be revealed by staining the tissue for the mitochondrial enzyme cytochrome oxidase^{12–15}. Here we report that V4 and V5 are connected with separate cytochrome oxidase-defined subregions of V2, suggesting that cortical pathways dealing with motion and colour perception are segregated in their passage through V2, and reinforcing evidence for functional specialization in the visual cortex^{16–18}.

In primates, the primary visual cortex V1 (area 17 or striate cortex) receives most of the visual input to the cerebral cortex and sends parallel outputs to several of the visual areas¹⁹ which, between them, comprise Brodmann's areas 18 and 19 of prestriate cortex. Because of this anatomical arrangement and the functional specialization in the prestriate areas^{16,17}, it was suggested that one function of V1 is to segregate different kinds of visual information relating to colour, form, motion and depth, and to distribute these to different prestriate areas for further processing¹⁹. Confirmation of this role of V1 has come from physiological studies in combination with cytochrome oxidase histochemistry. Tissue stained for this enzyme shows an array of 'blobs' in the upper layers of V1 in which are concentrated cells selective for wavelength but not orientation. In the more lightly staining 'interblob' regions, however, cells are normally not wavelength- but orientation-selective¹⁵. V2, the prestriate area bordering V1 (but occupying only part of area 18) receives a strong input from V1 (refs 20, 21) and, like it, contains several functional cell types^{16,22} and projects to several other prestriate areas^{23,24}. It, too, has a characteristic staining pattern for cytochrome oxidase, consisting of a series of stripes running orthogonal to the V1/V2 boundary^{12–15}. These are best visualized in tangential sections through the flat mounted occipital operculum (Figs 1c, 2a) where in most cases the dense stripes are alternately thick and thin and are separated by lighter regions more uniform in size, the 'interstripes'. Two of these three sets of repetitive subregions receive specific projections from V1, the thin stripes from the blobs and the interstripes from the interblobs, but the specificity (if any) of the input to the thick stripes from V1 remains unknown¹⁵.

Thus, the cytochrome oxidase-defined subregions of V1 and V2 are probably involved in segregated pathways of distinct functional characters and we sought to determine how the subregions of V2 project to the more uniformly specialized areas of prestriate cortex such as V5 and the V4 complex. These latter two areas differ profoundly in their functional characteristics: there is a total absence of wavelength-selective cells in V5, most

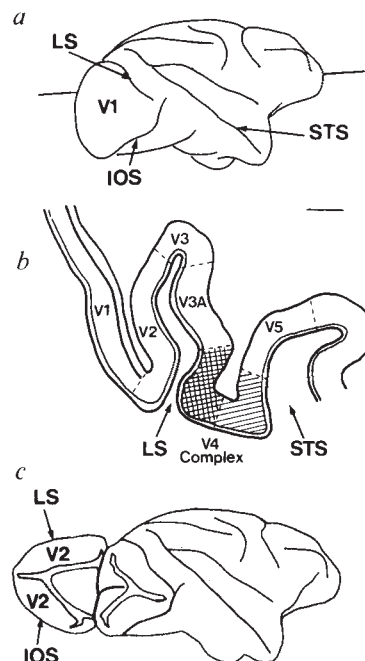


Fig. 1 Drawing of *a*, the surface of the left hemisphere of a macaque monkey brain; *b*, the lateral part of a horizontal section, taken at the level indicated in *a*, to show the locations of the three areas studied. The V4 complex (V4) consists of at least two areas with further subdivisions in each. In this study, the injections were made in the posterior part of the V4 complex (cross-hatched). *c*, Illustration of how the occipital operculum is removed and showing the relation of V2 to V1. LS, lunate sulcus; STS, superior temporal sulcus; IOS, inferior occipital sulcus. Scale bar, 2 mm.

cells being directionally selective^{1–3,25}, whereas V4 (which consists of at least two areas, each with probable subdivisions⁶) contains heavy concentrations of wavelength- and colour-sensitive cells but few that are selective for direction^{5,8,19}. Thus, by relating the distributions of cells projecting to V4 or V5 with the pattern of cytochrome oxidase stripes, it might be possible to associate specific subregions of V2 with specific visual functions.

Injections of wheat-germ agglutinin horseradish peroxidase (HRP) (0.1–0.3 μ l of a 4% solution) were made into area V5 in three monkeys and into V4 in five monkeys. After 48-h survival, the animals were anaesthetized and perfused, following standard procedures, after which the brains were sectioned at 50 μ m and pairs of adjacent sections stained for cytochrome oxidase²⁶ and reacted for HRP²⁷. The cytochrome oxidase-stained sections were drawn at $\times 30$ magnification under a drawing tube, with the boundaries of the cytochrome oxidase heavy zones and blood vessels marked. The adjacent HRP section, suitably magnified to compensate for its greater shrinkage, was superimposed according to the blood vessels and the positions of labelled cells and terminals added to the diagram. In this way, it was possible to determine the relation of the labelled cells to the cytochrome oxidase stripes in V2.

Where injections of HRP are made into V4 or V5, labelled cells and terminals in V2 are distributed in bands running orthogonal to the V1/V2 boundary and are very similar in disposition to the cytochrome oxidase stripes. The similarity is even more striking because, just as the cytochrome stripes show periodicities along their long axes²⁸, giving them a beaded appearance, so do the bands of labelled cells. Figures 2b and 3a show bands of label in corresponding locations in V2 following two separate V4 injections. In the former case the bands are quite narrow (0.7 mm) and well circumscribed, but in the latter they are broader (~ 1.5 mm) and rather more diffuse. The relationship of these bands to the cytochrome oxidase stripes is often difficult to determine because the pattern can be

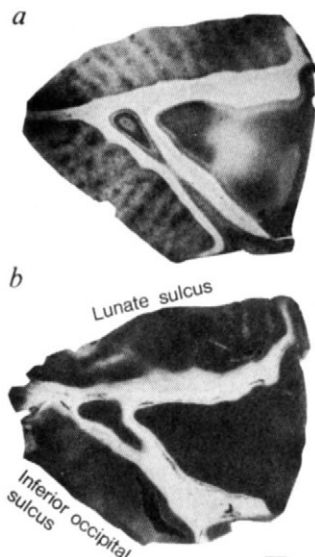


Fig. 2 *a*, Photomicrograph of a section taken through the flat-mounted occipital operculum and stained for cytochrome oxidase to show the thick and thin stripes and the interstripe zones in V2. The size of one cycle (centre-to-centre distance of two thin stripes) varies between 3.0 and 4.5 mm with a mean of 4.0 mm; the relative widths of the stripes also fluctuate, but are $\sim 2:1:1.5$ (thick:thin:inter). Not all macaque brains show such a clear pattern, especially in the lunate sulcus where the pattern is often complex and the thick and thin stripes are difficult to distinguish in individual sections. *b*, Dark-field photomicrograph of a similar section from a flat-mounted occipital operculum in an animal with an injection of HRP into V4. Note the striking similarity in the disposition of the labelled cells to that of the cytochrome oxidase stripes. The precise relationship of the two could not be determined in this brain, in which the cytochrome oxidase stain was unsatisfactory. Scale bar, 2 mm.

irregular, with alternate thick and thin stripes not easily discernible. Little could be learned from the cytochrome oxidase sections corresponding to the first case (Fig. 2*b*), in which the stain was technically poor; in the second case, where the staining pattern was irregular (Fig. 3*b*), superimposition of adjacent sections revealed that the labelled cells were centred over alternate stripes, and although overflowing into the interstripe zones, avoided the other set of stripes. That these were the thin stripes (those connected with the wavelength-selective blob of V1) was ascertained by tracing the pattern through a series of superimposed cytochrome oxidase sections, starting from those sections in which the stripes could be more clearly differentiated. This conclusion was reinforced by examination of another animal with a V4 injection, in which the two sets of stripes could be easily distinguished and in which a series of broad, if sparse, bands of label were again centred over thin stripes but avoided thick stripes (Fig. 3*d*). One further V4 injection again gave rise to the narrower bands of labelled cells and these were sited entirely in the interstripes. Four of these bands occupied 7.5 mm of cortex, a periodicity similar to that of the narrow bands of the brain in Fig. 2*b*, perhaps suggesting that these also might have a similar disposition with respect to the cytochrome oxidase stripes. This variation in the results obtained in V2 may reflect an inhomogeneity in V4 in relation to the distribution of wavelength and orientation selectivity^{2,19}. The injection sites, although all in the posterior portion of V4, may have involved physiologically dissimilar regions.

Following injections into V5, labelled cells in V2 again appear in bands running orthogonal to the V1/V2 boundary, but are now almost entirely confined to regions densely staining for cytochrome oxidase, which our evidence shows are the thick stripes. This was most clearly observed in the case of a brain sectioned horizontally (see Fig. 4*c*). The sections were drawn as described above and the information thus obtained was digitized along a

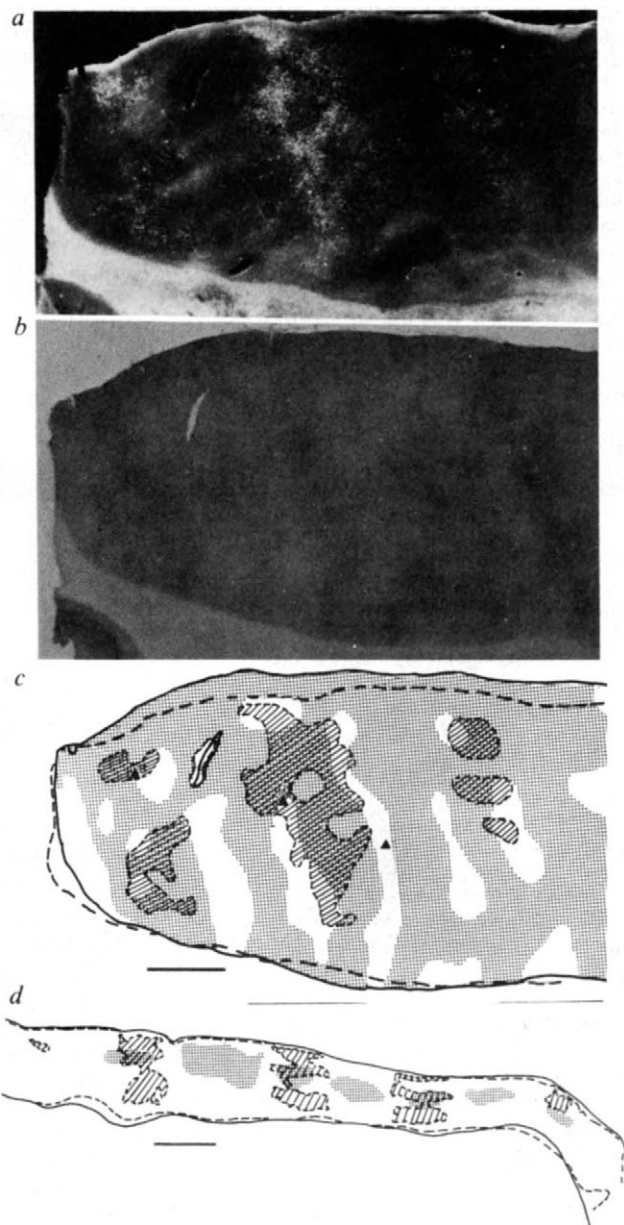
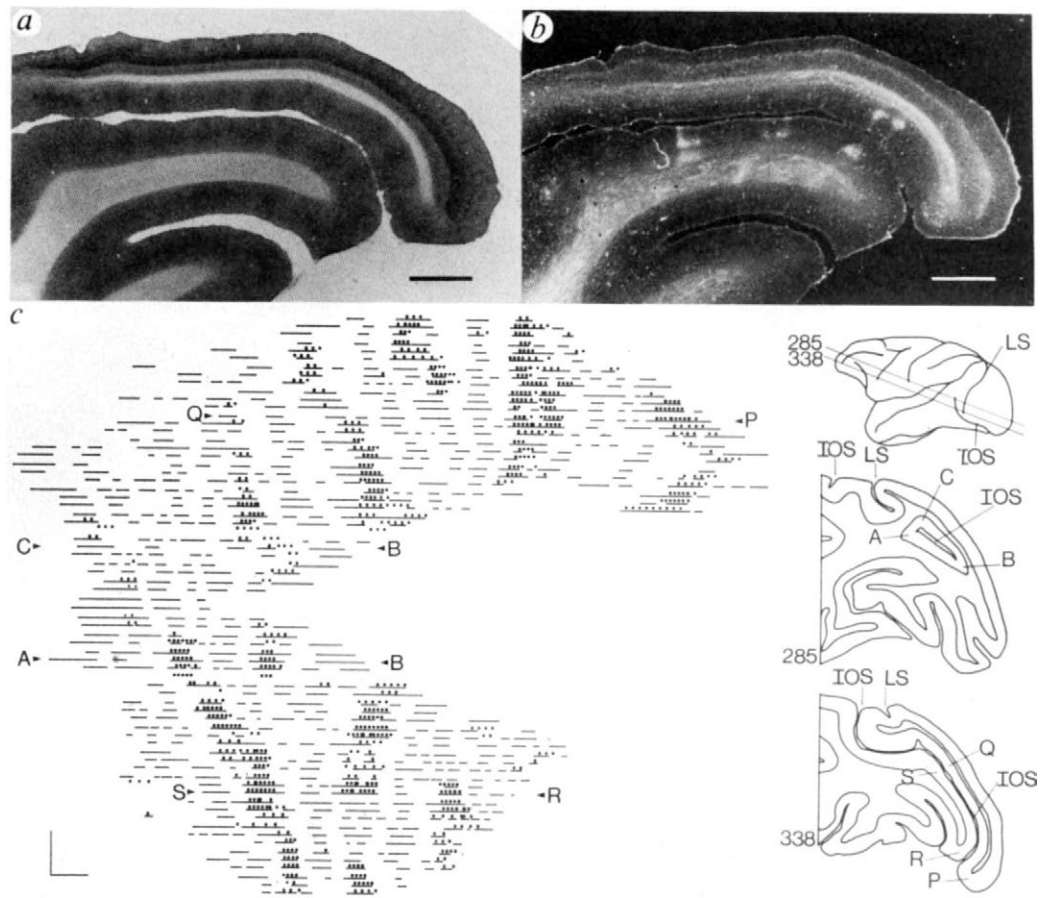


Fig. 3 *a*, Dark-field photomicrograph of the lunate sulcus from a flat-mounted operculum section to show the tangential distribution of labelled cells in V2 after an HRP injection into V4. *b*, Bright-field photomicrograph of a section next to the one shown in *a* and stained for cytochrome oxidase. *c*, Composite drawing, made from sections *a* (continuous outline) and *b* (interrupted outline) in which the two sections have been superimposed and areas of labelled cells (hatched) and dense stain (stippling) have been entered. The cytochrome oxidase distribution shown is also composite, drawn from superimposed images of section *b* and the other stained section next to *a*, a process that clarifies the pattern of stripes. Note that the labelled cells are centred over one set of stripes and skip alternate ones. We have not attempted to indicate more than one level of density for the distribution of either the labelled cells or of cytochrome oxidase, but it can be seen that both vary, not necessarily in parallel. For example, in the middle band of HRP-labelled cells (*b*) a roughly circular region of denser cytochrome oxidase staining coincides with a lower concentration of labelled cells. Unless traced over many sections, the stripes cannot, in this section, be identified as thick or thin. But *d* (a composite drawing made as for *c* but from sections from another brain with an HRP injection into V4), shows that the label is centred over the thin stripes and skips the thick ones. To align adjacent sections, as in *c* and *d*, we used blood vessels (three of which are indicated by triangles in *c*) and other landmarks to obtain the best fits, particularly because the shrinkage of the two sections is different in different directions.

Fig. 4 *a*, Brightfield photomicrograph of a horizontal section taken through the part of V2 lying in the inferior occipital sulcus and stained for cytochrome oxidase activity. *b*, Dark-field photomicrograph, taken from an adjacent section, following HRP injection into V5. *c*, Computer-aided reconstruction made from many pairs of sections similar to *a* and *b*. The information in each pair of adjacent sections was incorporated into a single drawing (as in Fig. 3c) and this information was then digitized and entered in the computer. The distribution of cells is shown as dots, and regions densely stained for cytochrome oxidase as broken lines. The reconstruction is symmetrical about the fundus of the inferior occipital sulcus; each line represents one horizontal section, and the diagrams of sections 285 and 338 with the marked points, A, B, C, P, Q, R and S indicate how the overall configuration relates to the anatomy of the cortex.



The cytochrome oxidase pattern is least clear where the stripes appear to be bifurcating, but elsewhere the appearance of alternate thick and thin stripes can be seen to emerge. LS, Lunate sulcus; IOS, inferior occipital sulcus. Scale bars: *a*, *b*, 2 mm; *c*, 1 mm for both axes.

'contour line' from the section and fed into a computer programmed to straighten and plot these lines to mimic the appearance that might have been obtained had the tissue been flattened and sectioned parallel to the cortical surface. The reconstruction reveals a number of irregularities, such as bifurcations in the stripes (which we have also observed in flat-mounted operculum cytochrome oxidase sections) but most of the stripes containing labelled cells are identifiably thick. This conclusion was in accord with the impression gained from those individual sections where alternating thick and thin regions of denser staining were visible, such as the lower bank of the inferior occipital sulcus in the section shown (Fig. 4*a*, the region between points 'R' and 'S' marked on its outline, labelled 338, in Fig. 4*c*).

In summary, we report here that alternating cytochrome oxidase stripes project to V4 and V5, our reconstructions suggesting that V4 receives input from the thin stripes and V5 from the thick, and that the interstripes also project to V4. Further evidence supporting functional distinctions between these subdivisions has come from our complementary physiological recordings (S.Z. and S.S., in preparation), in which we have made tangential penetrations running parallel to the stripes in the lunate sulcus. We have found that there is more variability in the responses recorded between penetrations than within any single penetration, and in several instances most of the units encountered in a single penetration, for a distance of over 1 mm, were either all orientation or all wavelength or all directionally selective. Where we have been able to associate penetrations with cytochrome oxidase stripes, we have found that interstripe cells are usually tightly tuned for orientation and are not wavelength-selective; most wavelength-selective cells, some of them double opponent, are found in or at the borders of thin stripes and are not orientation-selective. The frequency of occurrence of orientation selectivity is at an intermediate level in thick stripes, but these penetrations have the highest incidence of

directional selectivity. Thick stripe cells, whether or not they are orientation-sensitive, generally respond equally well or better to spots of light moved in any direction than to long bars, unlike the orientation-selective cells of the interstripes, whose responses to spots are poor.

Thus, both anatomical and physiological evidence indicates that any operations performed in V2 must be performed separately for the different visual submodalities. This does not eliminate any interplay between the different pathways, given both local connectivities and that the outflow from V2, at least to V4, is not absolutely restricted to the territory of the thin stripes. But the segregation of pathways through V2 is much the more impressive and further reinforces the earlier evidence for functional specialization within the visual cortex¹⁷.

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Note added in proof: We have since found that V3, which has no wavelength-selective cells, also receives from cells in the thick cytochrome oxidase stripes of V2.

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Complex-unoriented cells in a subregion of primate area 18

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In primates, both the primary and secondary visual cortical areas can be subdivided histologically by staining for the mitochondrial enzyme cytochrome oxidase^{1–3}. In the primary visual cortex (area 17, the first cortical receiving area for visual information) these histological differences correspond to functional subdivisions^{1–6}, cytochrome-dark regions being concerned with information about colour and cytochrome-light regions concerned with form. Here we report that the second visual area, area 18, which receives its main cortical input from area 17 (refs 7, 8), similarly has functional subdivisions that correspond to the cytochrome oxidase staining pattern. In area 18 the segregation between form and colour is maintained, reinforcing our notion that form and colour information follow parallel pathways. The specific differences between cells in areas 17 and 18 suggest that a possible step in hierarchical information processing is spatial generalization, analogous to the difference between simple and complex cells.

In area 17 the cytochrome oxidase staining pattern consists of small dark-staining polka-dots (blobs) on a lighter-staining background^{1,2}. Cells in the blobs are not orientation-selective and at least half are colour-coded, whereas most cells in the inter-blob regions are orientation-selective with only a small number colour-coded⁶. In area 18 the dark-staining regions are much coarser and form stripes, ~0.5–1 mm wide, which run perpendicular to the 17/18 border and extend the full width of area 18 to its anterior border (Fig. 1; refs 3–5). In some macaques and in most squirrel monkeys, the dark stripes are generally of two types, alternating thin and thicker, slightly lighter stripes. Although the thick stripes are not always easy to distinguish from the thin stripes by cytochrome oxidase staining alone, they differ anatomically in that they have a different laminar distribution of thalamic inputs⁴. Thus, area 18 seems to consist of at least three types of subregions: thin stripes, thick stripes and, in between, much lighter interstripes.

We have recorded from 1,019 cells in area 18 of 11 young adult female *Macaque fascicularis* monkeys and 7 adult female *Saimiri sciurius* (squirrel) monkeys. The recording apparatus was as described previously⁶. We made electrode penetrations perpendicular to the cytochrome oxidase stripes as nearly parallel to the surface as possible.

It was clear that area 18, like area 17, is physiologically heterogeneous but with a much coarser periodicity. Figure 2 shows the results from one macaque monkey, in which we recorded from 9 mm of area 18; almost all of the penetration appears in this single section. The figure indicates that we passed through four cytochrome oxidase-dark regions. Two of these stripes (the second and fourth) consisted exclusively of cells that entirely lacked orientation selectivity, whereas almost all the cells in the other two stripes and in the interstripe regions were orientation-selective. We found such long stretches of

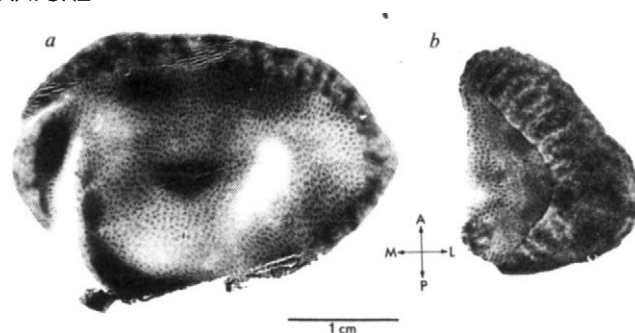


Fig. 1 Sections of monkey visual cortex stained for cytochrome oxidase. *a*, Section from a macaque monkey. Most of the section is in area 17 except for a 0.5-cm-wide strip of area 18 at the top. *b*, Section from a squirrel monkey. Area 17 is to the left with the fine polka-dot pattern and area 18 is to the right with a coarser-striped pattern. The alternation of thick and thin stripes is clearest near the top of the squirrel monkey section and is not apparent in the macaque.

unoriented cells in both macaques and squirrel monkeys (211 cells), always in cytochrome oxidase-dark regions. In the macaques it was not usually possible to say whether the dark stripes containing unoriented cells were thick or thin, but they usually alternated with stripes containing oriented cells, as in Fig. 2. In the squirrel monkeys the unoriented cells were clearly in the thin stripes. All these results are consistent with our earlier observation that the blobs in area 17 and thin stripes in area 18 are interconnected.

In macaques, about two-thirds of the unoriented cells showed colour opponency; in the squirrel monkey the proportion of colour-coded cells was much smaller. Of the colour-coded cells, a few had colour opponent centres and no antagonistic surrounds (for example, red-on centre, green-off centre, Type II; ref. 6), but most had some form of double-opponent colour coding (for example, red-on centre, red-off surround, green-off centre, green-on surround). Similar to blob cells in area 17, the unoriented cells in area 18 (whether or not they are colour-coded) responded more strongly as spot size was increased up to an optimum size beyond which the responses declined. What was different about many of these cells, compared with the blob cells in area 17, is that they responded to a spot of optimum size regardless of where it was positioned, over an area that was itself many times larger than the optimum spot. Thus, in these cells a spot large enough to cover the entire activating region was usually ineffective.

An example of one such colour-coded cell is shown in Fig. 3. It gave on responses to small red spots, off responses to small blue or green spots and no responses to white or to yellow spots (580 nm). In response to an optimal spot, 0.5° in diameter, the cell gives on responses to red and off to blue or green throughout an activating region 3.5° in diameter, maximum near the centre and falling off gradually with distance; increasing the spot beyond 0.5° resulted in a fall-off in responses (Fig. 3*b, c*). When we shone a small red and a small blue spot simultaneously in the same position (giving a yellow spot), the cell gave little or no response. We did not try spatially to separate red and blue spots with this cell (but in other similar cells, simulating with a small red spot and a small blue spot side-by-side within the activating region gave mixed on/off responses). This cell was located in layer 5 of a cytochrome oxidase-dark stripe in area 18 of a macaque.

We also observed cells, similar but not colour-coded, some giving on and others off responses regardless of wavelength over an activating region larger than the optimum spot size. Baizer *et al.*⁹ have described 'spot cells' in area 18 that are similarly unoriented, not colour-coded and respond best to a small spot anywhere in a large receptive field. We term all of these cells, whether or not they are colour coded, 'complex-unoriented', because they differ from ordinary centre-surround cells in a manner analogous to the difference between oriented simple and complex cells¹⁰. (The distinction lies in the permissible

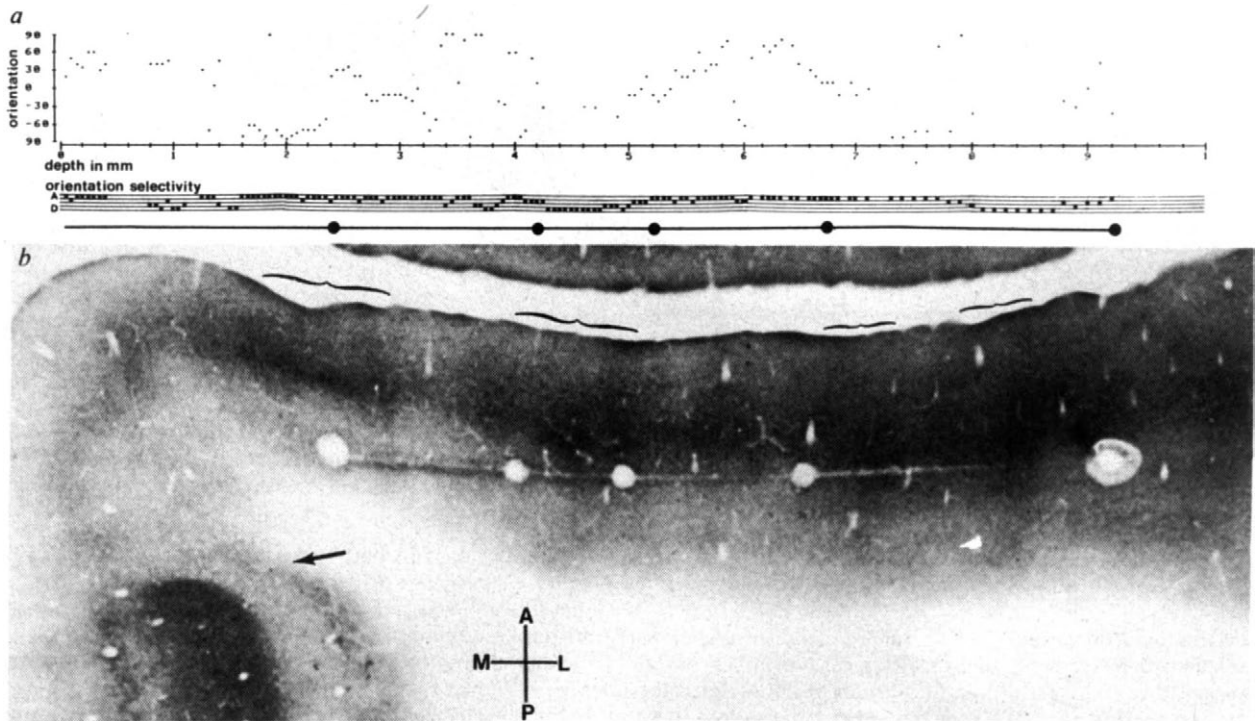


Fig. 2 *a, b*, Reconstruction of an electrode track in area 18 of macaque. *b*, Transverse section through area 18 just anterior and parallel to the area 17/18 border. Part of the border is visible at the left edge of the section (arrow). The space shown just above area 18 is the lunate sulcus; the midline is on the left. The electrode track, most of which can be seen together with five electrolyte lesions, runs from left to right. The section was stained for cytochrome oxidase²² and the cytochrome-dark regions are indicated by brackets. *a*, From above downwards, preferred orientation (0°, vertical); orientation selectivity (A, well oriented, D, unoriented); and the location of the electrolyte lesions. Optimum orientation and orientation selectivity were judged subjectively by listening to the response of the cell to slits of light generated by a hand-held slide projector. Observations were made every 0.05 mm. Note the correspondence between two of the cytochrome-dark regions (between second and third lesions and just before the final lesion) and the absence of orientation selectivity. (At these points no orientation could be plotted.) The entire track after the first 0.5 mm was below layer 4.

variation in position; see ref. 10.) In both cases, although the size of the region of the visual field in which the higher level cell can be activated is larger (in our complex-unoriented cells, from two to several times the diameter), the optimum stimulus size for a given eccentricity remains the same, as if an 'or-gate' operation had been performed on many cells with similar receptive fields scattered over an area larger than each separate receptive field. Thus, although resulting in a loss of precision of localization, complexification need not lead to a loss of resolution or acuity, because at a given eccentricity the average optimum stimulus size, such as line width or spot diameter, shows no such increase. The increase in receptive-field size reported in several pre-striate areas¹¹⁻¹⁴ is presumably, at least in part, the result of such transformations. The occurrence of similar operations in different systems suggests that similar circuits are used, which would doubtless simplify the problem of building the cortex. Despite the frequency with which this operation seems to occur in the visual system, its role in information processing is still a matter of conjecture. (See page 285 of this issue.)

Some of the unoriented cells we found in area 18 were not complex and seemed the same as cells that we have observed in area 17. We are now trying to determine whether the different orders of unoriented cells in area 18 are segregated into different layers, as any sequence of unoriented cells tended to contain either ordinary or complex unoriented cells. In area 17, however, we have not seen complex-unoriented cells. The transformation of colour information in going from area 17 to 18 might be predicted to involve the acquisition of orientation selectivity, but this seems not to be so.

In the squirrel monkey it is clear that the regions in area 18 containing complex-unoriented cells are the same regions, the thin stripes that we previously found anatomically to be connected to the blobs in area 17. These blobs contain unoriented, often colour-coded cells⁶. In macaque monkeys the histological

distinction between two types of cytochrome-dark stripes is often less clear, but, as shown in Fig. 2, only some of the stripes (usually alternate ones) are also characterized by colour-coded unoriented cells.

We have also studied the characteristics of the cells in the other stripes: cells in both the interstripes and the thick stripes show orientation selectivity and are not colour-coded. The light-staining interstripes in area 18 are reciprocally connected with the inter-blob regions of area 17, which contain oriented non-colour-coded cells⁶; the connections of the thick stripes are not yet known, but they clearly receive input from area 17 (see Fig. 30 of ref. 6). The most striking characteristic of cells in the interstripe regions is that over half of them are end-stopped¹¹, responding optimally to slits of a particular length and less well to longer slits. This suggests that the system that in area 17 carries information about the orientation of edges might next acquire information about their degree of curvature. In the thick stripes the cells seem to be concerned primarily with binocular disparity: many fail to respond to either eye alone but do respond if the eyes are aligned with a particular disparity, corresponding either to near, far or peaking at zero disparity. DeYoe and Van Essen¹⁵, by injecting two different retrograde tracers into the pre-striate visual areas MT and V4 of macaques, have found that in area 18 alternating cytochrome-dark stripes project to MT and to V4. The results of Shipp and Zeki¹⁶ in the accompanying paper confirm these observations. Given the high proportion of colour-coded cells reported in V4 (ref. 14), the V4-projecting stripes would be expected to be the ones to receive input from the blobs in area 17 and contain unoriented, often colour-coded cells: in a squirrel monkey these would be the thin stripes. Our finding that the thick stripes contain orientation-selective cells is consistent with their involvement with some system other than the colour system; they could correspond to the MT-projecting stripes described by DeYoe and Van Essen¹⁵. The segregation in area 17 of two systems, one apparently concerned with form

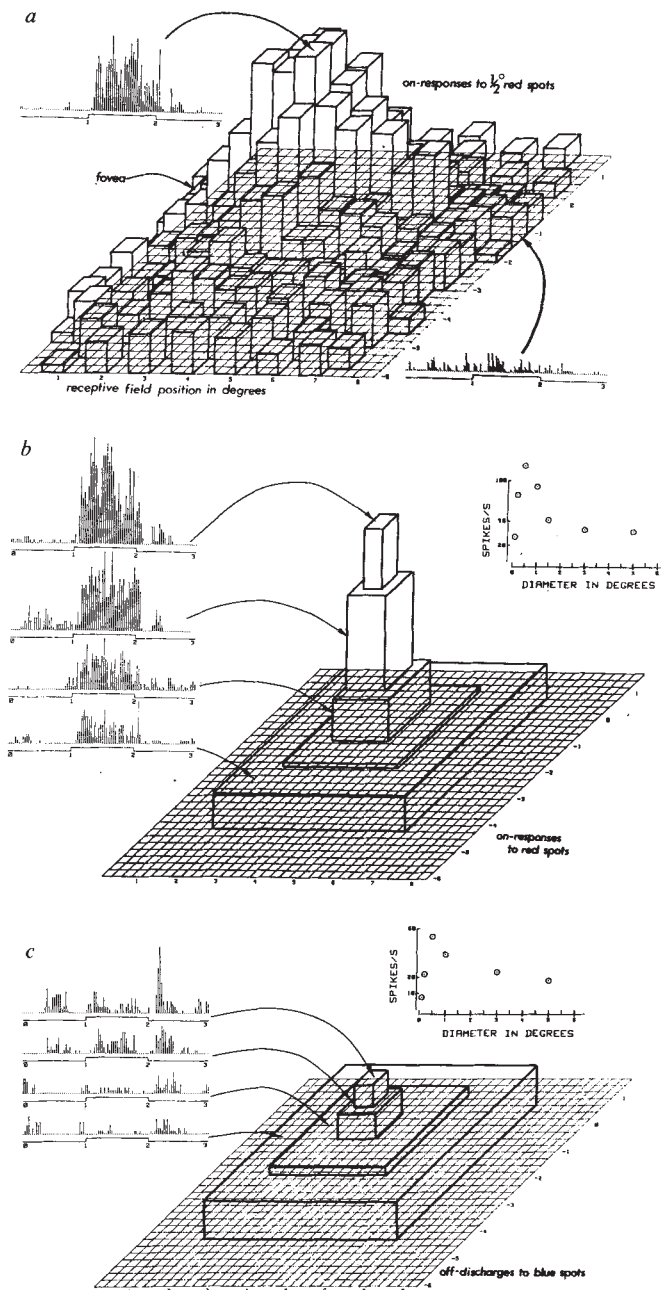


Fig. 3 Responses of a complex double-opponent cell in area 18 of a macaque monkey to red and blue spots of light, varying size and position of spot. The grid represents the visual field, marked off in degrees, with the fovea at (0, 0). The red and blue spots were produced by Kodak Wratten filters (29 and 47B) placed in front of a slide projector. **a**, Three-dimensional plot illustrating responses to red spots of optimal size, 0.5° in diameter. The plot is made over a region 8×8°, centred 5° lateral and 2.5° below the fovea. Responses were obtained over an area occupying 3×3°, outside which the bars represent spontaneous background activity. Bars represent means of five responses to stimuli of 1 s duration. The histogram for the area giving the peak response, shown in the inset on the upper left, represents 85 impulses during this 'on' period. The lower right inset shows one of the histograms for a region giving little or no response above the background firing rate. **b**, Diagram of on-responses to red light spots of various sizes. Each histogram is a mean of five stimuli. The three-dimensional graph illustrates the responses to the optimum size and to larger stimuli; the inset on the left shows the histograms obtained for four of these five spots. The upper right inset shows the entire curve of response versus spot size. Note that the 3.5 and 5° stimuli evoked responses that were barely visible above background firing levels, even though these covered the area over which 0.5° spots gave vigorous responses. **c**, Same cell, off-discharges to blue spots of different sizes.

and the other with colour, evidently persists into area 18 and beyond. That a similar segregation occurs in humans is apparent from reports of subjects with local cortical damage who experience loss of colour vision but retain normal form, movement and depth perception¹⁷⁻²¹.

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Induction of regulatory T-lymphocyte responses by liposomes carrying major histocompatibility complex molecules and foreign antigen

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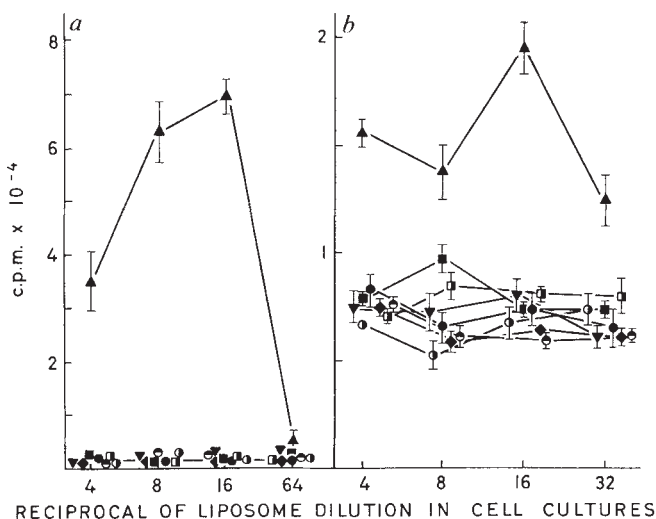
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Regulatory (helper and suppressor) T lymphocytes become activated only when foreign antigen is presented to them on the surface of antigen-presenting cells (APC), together with class II major histocompatibility complex (MHC) molecules (heterodimers of polypeptides of 28,000 and 35,000 relative molecular mass)¹⁻⁴. Once activated by a certain foreign antigen—MHC combination, T cells react to the same antigen only in combination with the same MHC molecule, a phenomenon termed MHC restriction of T-cell recognition (reviewed in refs 1, 5). Studies of the mechanisms involved in antigen presentation and MHC restriction have been hampered mainly by the virtual impossibility of inducing T-cell responses in the absence of APC. We describe here the production of synthetic lipid vesicles with inserted class II MHC molecules and a protein antigen coupled covalently to the lipid. These liposomes are shown to stimulate cloned helper T cells and T-cell hybridomas in an antigen-specific, MHC-restricted manner in the absence of APC. Thus, the recognition of foreign antigen together with class II MHC molecules seems to be the only signal required for the activation of antigen-primed regulatory T cells. Furthermore, 'processing' of antigen by APC is not essential for its recognition by T cells.

To determine the minimal requirements of T-cell activation, we constructed liposomes carrying a foreign protein antigen, or class II MHC molecules, or both, and tested whether these liposomes could activate antigen-specific class II-restricted T cells in the absence of APC. Class II MHC molecules were isolated from lipopolysaccharide-stimulated spleen cells. The membrane proteins were solubilized with 50 mM β -octyl gly-

Fig. 1 Liposome-bound antigens induce the proliferative response of an ovalbumin (OVA)-specific, A^b-restricted T-cell clone (a) and IL-2 production by a bovine insulin (BI)-specific A^b-restricted T-cell hybridoma (b). Liposomes with the following proteins were used: ▲, a, OVA + A^b; ■, a, OVA; ●, a and b, A^b; ▼, mixture of liposomes with: a, OVA + A^b; b, BI + A^b; □, a, OVA + A^k; b, BI + A^k; ○, a, BI + A^b; b, OVA + A^b; ●, a, free OVA + A^b; b, free BI + A^b; ◆, liposomes without protein. Each point represents the arithmetic mean $\pm$ s.d. (vertical bars; not shown where too small to be represented graphically) of triplicate cultures. Control values (c.p.m. $\pm$ s.d.) for the proliferative response of OVA-7 (a) and for the IL-2 production by hybridoma 52H10F11 (b): feeder cells, a (C57BL/6), 654 ± 124 ; b (C57BL/6), $6,505 \pm 522$; antigen + feeder cells, a (OVA $100 \mu\text{g ml}^{-1}$ + C57BL/6), $202,066 \pm 12,019$; b (BI $200 \mu\text{g ml}^{-1}$ + C57BL/6), $140,381 \pm 1,727$; control antigen + feeder cells, a (BI + C57BL/6), 657 ± 366 ; b (OVA + C57BL/6), $6,976 \pm 611$; antigen + control feeder cells, a, not done; b (BI + B10.A), $5,162 \pm 542$.

Methods. Clone OVA-7 of C57BL/6 origin (M. H. Schreier, unpublished data) was precultured with IL-2 and without feeder cells for 6 days, washed and distributed in 0.2-ml wells (3×10^4 cells per well), cultured in the presence of liposomes for 3 days (without IL-2 and feeder cells), and proliferation was measured by ^3H -thymidine incorporation²⁶. Hybridoma 52H10F11 (BI-specific, A^b-restricted; B. Huber, unpublished data) was cultured (5×10^5 cells ml^{-1}) in the presence of liposomes for 2 days. Supernatants were then collected and tested for their capacity to induce proliferation of the IL-2-dependent cell line CTLL²⁷ (5×10^3 cells per well, 1 day's culture followed by a 16-h pulse with $2 \mu\text{Ci}$ per well of ^3H -thymidine). Culture medium was α minimal essential medium supplemented with 10% fetal calf serum, antibiotics, L-glutamine and 3×10^{-5} M 2-mercaptoethanol. Liposomes were prepared by the dialysis method^{7,28}. They consisted of dipalmitoylphosphatidylethanolamine (DPPE), 10% of which was SPDP-modified (the efficiency of modification was 1–1.5%). Liposomes were allowed to react with a large excess of SPDP-coupled OVA and BI, respectively (2.5 mg to 12.5 mM lipid). The antigen-coupled liposomes were purified by sucrose gradient centrifugation (5–45% flotation gradient, 100,000g, 46 h). The density of antigen on liposome was monitored by the position of the liposome band in the gradient. Class II MHC molecules were purified by affinity chromatography using monoclonal antibodies 17.227.R7 (ref. 29) for the A^b, and 10.2.16 (ref. 30) for the A^k molecule.



copyranoside in 10 mM Tris-buffered saline solution. Further purification was performed by affinity chromatography according to the procedure described by Turkewitz *et al.*⁶, with the modification that β -octyl glycopyranoside was used as a detergent throughout. Monoclonal class II-specific antibodies used for the affinity columns were coupled with Sepharose-4B by the cyanogen bromide method followed by fixation with glutaraldehyde. The one-step affinity chromatography yielded highly enriched class II preparations with minor contamination by other proteins, as judged by SDS-polyacrylamide gel electrophoresis (data not shown). The class II molecules were inserted into liposomes made of synthetic lipids as described elsewhere^{7,8}. Lipids in the liposomes were activated by thiolation with *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) via a disulphide bond as described previously^{9,10}. The foreign protein antigens used were attached to these SPDP-activated lipids. The liposomes thus obtained carried class II MHC molecules inserted into the lipid bilayer by their hydrophobic transmembrane portion, and protein antigens covalently linked to the lipids. The vesicles were further purified by ultrafiltration, ultracentrifugation and in some experiments by flotation on a sucrose gradient. Flotation experiments were also performed to monitor the efficiency of reconstitution of MHC molecules into, and coupling of antigens to, the liposomes.

Figure 1 demonstrates that only the liposomes carrying both the priming antigen and the appropriate MHC molecules are able to stimulate proliferative response of an ovalbumin-specific, A^b-restricted T-cell clone, and interleukin-2 (IL-2) production by an insulin-specific, A^b-restricted T-cell hybridoma, in the absence of APC. Thus, the recognition of antigen + MHC molecules alone seems to provide a sufficient signal to induce these two kinds of responses of helper T cells. Note that, for a response to occur, the antigen and MHC molecules must be incorporated into the same liposome, indicating that the receptor structure of T cells recognizes these two moieties together. The finding that mixtures of antigen-bearing and MHC-bearing liposomes are not stimulatory, together with the results of other control groups of the experiments (MHC-bearing liposomes presented together with free antigen; antigen-bearing liposomes alone) makes it very unlikely that the liposomes are taken up and re-presented by residual APC or by the T cells themselves.

Further evidence to rule out this possibility will be presented elsewhere (P.W., Z.A.N. and J.K., in preparation).

Our data extend previous results from several laboratories that demonstrated that secondary cytolytic T-cell responses and secondary mixed lymphocyte reactions can be stimulated by cell-free antigens, although in these experiments the possibility that APC contributed to the T-cell activation could not be excluded^{11–18}. Our results also agree with a recent report by Malissen *et al.*¹⁹ that the expression of transfected class II MHC genes seems to be the only requirement to convert a cell into an APC. The fact that intact protein molecules, when coupled to lipid, can activate T cells indicates that extensive processing is not required to render a protein immunogenic for T cells. Thus, although antigen degradation certainly takes place in macrophages and perhaps also in other cell types^{20,21}, and although the existence of T-cell clones with a preference for partially digested antigens has been demonstrated^{22,23}, the notion that regulatory T cells are capable of recognizing only degraded (or processed) antigen^{24,25} is not generally valid.

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Developmental acquisition of Ca^{2+} -sensitivity by K^+ channels in spinal neurones

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A developmental change in the ionic basis of the inward current of action potentials has been observed in many excitable cells¹. In cultured spinal neurones of *Xenopus*, the timing of the development of the action parallels that seen *in vivo*^{2,3}. *In vitro*, as *in vivo*, neurones initially produce action potentials of long duration which are principally Ca-dependent; after 1 day of development the impulse is brief and primarily Na-dependent³⁻⁵. At both ages, however, both inward components are present⁴⁻⁶ and the mechanism underlying shortening of the action potential is unknown. One possibility is that the outward currents change during development. Using the patch-clamp technique⁷, we have recorded single K^+ -channel currents in membrane patches isolated from the cell bodies of cultured embryonic neurones⁸. The unitary conductance of one class of K^+ channels was ~ 155 pS and depolarization increased the probability of a channel being open. Neither conductance nor voltage dependence seemed to change with time in culture; in contrast, the Ca^{2+} -sensitivity of this K^+ channel increased. In younger neurones, Ca^{2+} -sensitivity was greatly reduced or absent, whereas in more mature neurones, the activity of this channel was Ca-dependent. Such a change could account for the shortening of the action potential duration by increasing the relative contribution of outward currents.

Single-channel currents of *Xenopus* neurones were recorded at two stages of development in culture: early (7-11 h) and mature (22-43 h). All recordings were made from inside-out excised patches at room temperature. In most cases, K^+ was the predominant monovalent cation in both pipette and bath solutions. Channel activity during prolonged depolarization was characterized by bursts of openings interrupted by brief closures and followed by long closed intervals. The unitary conductance was estimated from the single-channel current/voltage ($I-V$) relationship. After 1-2 days in culture, a channel with a conductance of 156 ± 2 pS (mean $\pm$ s.e.m.) was observed in 15 patches. Unitary conductance was independent of membrane potential (± 50 mV), of internal Ca^{2+} concentration ($[\text{Ca}^{2+}]_i = 0.5-100 \mu\text{M}$), and of external Ca^{2+} concentration ($[\text{Ca}^{2+}]_o = 0-0.5$ mM). Similarly, at early ages a channel with a unitary conductance of 154 ± 2 pS was observed in nine patches; conductance was independent of calcium concentration and membrane potential.

At both ages these channels selected primarily K^+ ions. Currents reversed direction at the K^+ equilibrium potential (E_K), both in symmetrical K^+ and when Tris^+ was partially substituted for internal K^+ , shifting E_K to $+17.5$ mV ($n = 6$ patches at 1-2 days; $n = 6$ patches at 7-11 h). However, when recordings were made in saline solution with Na^+ as the predominant extracellular cation and K^+ primarily on the intracellular face ($E_K = -75$ mV), unitary currents reversed at an extrapolated potential of ~ -50 mV ($n = 5$ patches, 1 day in culture). If the channel is

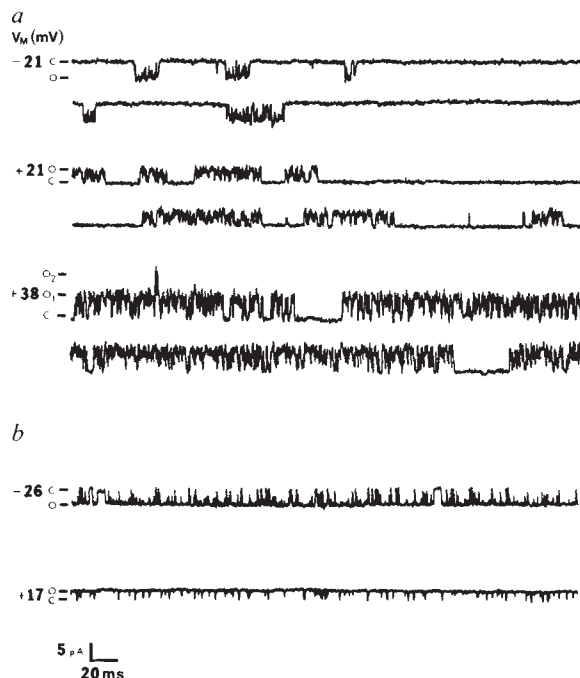


Fig. 1 Calcium- and voltage-sensitivity of K^+ channels in an 'inside-out'⁷ patch excised from a neurone after 23.5 h in culture. *a*, Single-channel currents recorded with $1 \mu\text{M}$ Ca^{2+} at the intracellular surface. Openings occurred in bursts separated by long quiescent periods: O, open; C, closed. Burst durations increased and inter-burst intervals decreased with depolarization. Single-channel conductance was 158 pS. *b*, Increasing the $[\text{Ca}^{2+}]_i$ to $10 \mu\text{M}$ increased burst duration and decreased inter-burst closed intervals. At $E_m = +17$ mV, channels were almost continuously open. Same patch as in *a*. Multiple simultaneous openings occurred at depolarized potentials, indicating that a minimum of three channels were active in the patch (not illustrated). Activity of smaller-amplitude channels was not observed. During a burst there were many brief closures which were poorly resolved. Some of these apparent closures may have been only partial, to a lower-conductance sub-state. Membrane potential was varied by resetting the holding potential across the patch. Data were filtered at 4 kHz, sampled at 50- μs intervals, and recorded in 460.8-ms segments.

Methods. Neurones were cultured from dissociated *Xenopus* neural plates (Nieuwkoop and Faber stage 15)¹⁶ as described previously^{3,4}, and single-channel currents were recorded at room temperature using established techniques^{7,17,18}. Data were analysed for current amplitudes by computer^{17,18}; the threshold for event detection was 50% of the mean amplitude. Burst duration and inter-burst intervals were measured by hand from a chart record of the data. Unless otherwise stated, the saline solution in the pipette and in the bath comprised (in mM): 150 KCl, 10 or 1 EGTA (results were indistinguishable), 5 HEPES, pH 7.8. Free internal Ca^{2+} concentrations of 0.5, 1, 10, 25 and $100 \mu\text{M}$ were achieved by adding CaCl_2 to the bath saline. These concentrations were calculated assuming purity of the EGTA (but see ref. 19). Bath volume was 0.2 ml; bath changes were achieved by washing the patches with ≥ 25 vol. saline.

assumed to be permeable to Na^+ as well as K^+ , the relative Na^+/K^+ permeability, calculated from the Goldman-Hodgkin-Katz equation, was 0.08-0.1. An alternative explanation for the apparent imperfect selectivity is that the $I-V$ relationship was nonlinear in the area of E_K .

Embryonic *Xenopus* neurones exhibited a marked developmental difference in the effect of internal calcium on the activity of this channel. At 1-2 days, activity was sensitive to $[\text{Ca}^{2+}]_i$ (Fig. 1). Opening was not detected when free $[\text{Ca}^{2+}]_i$ was estimated to be $< 10^{-10}$ M. Raising the $[\text{Ca}^{2+}]_i$ from 1.0 to $10 \mu\text{M}$ or from 0.5 to 25 or $100 \mu\text{M}$ substantially increased channel activity ($n = 12$ of 14 patches): both burst duration and the number of simultaneous openings increased, while the inter-burst intervals decreased. In the experiment shown in Fig. 1, raising the $[\text{Ca}^{2+}]_i$ from 1.0 to $10 \mu\text{M}$ increased the mean burst

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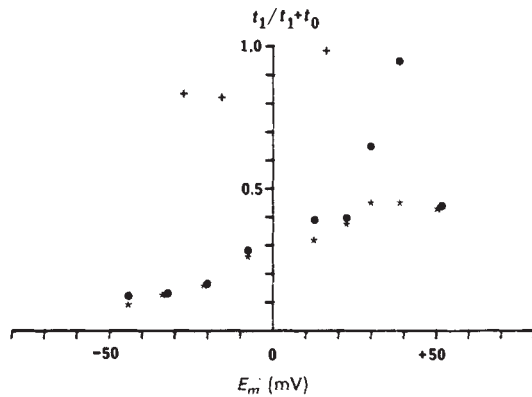


Fig. 2 The fraction of time for which one channel was open changed with membrane potential, $[Ca^{2+}]_i$, and the length of time after a change in membrane potential. This fraction was calculated as t_1/t_1+t_0 in which t_1 is the time for which a unitary level of channel activity was observed, t_0 the time in which no activity was observed. In $1 \mu M$ calcium, the fraction of time spent open increased with depolarization (●). Increasing the $[Ca^{2+}]_i$ to $10 \mu M$ increased this fraction several-fold (+). The fraction decreased during the recording in a time- and voltage-sensitive manner, presumably as a result of inactivation. In $1 \mu M$ calcium, the fraction was compared between the first 460.8 ms (●) and the section 2.9–3.4 s into the record (*); a large reduction was observed at depolarized voltages. At 38.1 mV, this reduction occurred within the first second of recording; at less depolarized voltages, the reduction was slower as well as smaller. The low initial activity seen at +50.3 mV probably results from inactivation during the ~1-s delay between setting the membrane potential and the start of recording. Ending depolarization, then re-stepping to positive potentials regenerated high levels of activity. Data are from the patch shown in Fig. 1.

duration almost 15-fold; the mean inter-burst interval decreased ~30-fold (at membrane potential (E_m) = +17 mV). This resulted in a large increase in the fraction of total time for which a channel was open (Fig. 2). Different results were obtained from 2 of the 14 patches: in one case, channel activity appeared to be insensitive to change in the $[Ca^{2+}]_i$; in the other, few openings were observed until $[Ca^{2+}]_i$ was raised to $100 \mu M$, but reducing the $[Ca^{2+}]_i$ subsequently failed to decrease the frequency of opening.

In contrast to the Ca^{2+} -sensitive activity observed in patches from mature neurones, channel activity in patches excised from early neurones was insensitive to changes in $[Ca^{2+}]_i$ ($n=9$ patches). Currents were observed with free $[Ca^{2+}]_i < 10^{-10} M$ (Fig. 3), and increasing the $[Ca^{2+}]_i$ to $100 \mu M$ did not change the fraction of time for which a channel was open. In six additional patches no channel activity was observed in Ca^{2+} -free saline; raising the $[Ca^{2+}]_i$ to $100 \mu M$ did not induce activity. Extracellular calcium had no effect on channel activity of either early or mature neurones.

Activity at both ages was voltage-dependent. Figure 2 shows that the fraction of time for which a channel was open increased with depolarization. In addition, the mean inter-burst closed time declined and the mean burst duration increased, suggesting that the increase in open time of the channels was due both to an increased probability of opening to a burst and to a decreased probability of closing, once opened. In other patches, the fraction of time spent open showed sigmoidal increases with depolarization similar to that in Fig. 2; the half-maximal value was attained at ~+20 mV in all cases. The frequency of occurrence of bursts showed comparable voltage-dependence.

Most patches also possessed a lower-conductance K^+ -selective channel: $94 \pm 3 pS$ at day 1 ($n=4$ patches); $93 \pm 1 pS$ at 8.5–10.5 h in culture ($n=4$ patches; Fig. 3). Opening of this channel was not correlated with that of the larger channel. The fraction of time for which the channel was open and the frequency of bursting were unaltered by changes in $[Ca^{2+}]_i$ but increased with depolarization. The half-maximum of the fraction

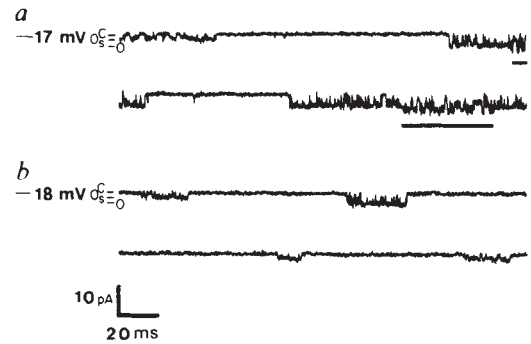


Fig. 3 Ca^{2+} -insensitive K^+ currents recorded from an inside-out patch of membrane excised from a neurone at 8.5 h. *a*, Single-channel currents at -17 mV were recorded in the absence of intracellular calcium; *b*, single-channel activity at -18 mV in $100 \mu M$ calcium. These records also illustrate the presence of a smaller-amplitude K^+ channel which was Ca^{2+} -insensitive at all ages and appeared to be independent of the ~155-pS channel studied here. O, opening of a 155-pS channel; O_s, opening of a smaller-conductance K^+ channel; C, closed. The dark bars denote regions of simultaneous opening of both the large- and small-amplitude channels. Data were filtered at 2.5 kHz and digitized at 100-μs intervals.

of time spent open occurred at ~0 mV. Although lower conductances have been reported in other neural preparations^{8,9}, this channel may represent a delayed rectifier.

The Ca^{2+} -sensitive K^+ currents observed in these neurones at 1–2 days in culture appear to be similar to those of other preparations. Unitary conductances of 100–220 pS^{8,10–12} at room temperature, calcium- and voltage-independence of the conductance¹⁰, and calcium- and voltage-sensitive changes in bursting^{10,13,14} have been reported, but inactivation was not prominent^{10,13}.

The results reported here suggest that the acquisition of calcium-sensitivity accounts in part for the developmental disappearance of the Ca^{2+} -dependent plateau of the action potential. The lack of age-dependent changes in unitary conductance, K^+ -selectivity and voltage-dependence of activation suggests that the Ca^{2+} -sensitive and -insensitive channels represent a single class of channel, the gating mechanism of which is developmentally modified. Different sensitivities could be produced through mechanisms ranging from activation of a multi-gene family to post-translational modification. The infrequent presence of Ca^{2+} -insensitive channels in older neurones could indicate that these channels are inserted into the membrane in a form having reduced sensitivity and are then modified, or that a population of Ca^{2+} -insensitive channels persists into late stages of development. In view of recent results with the acetylcholine receptor¹⁵, it would be of interest to see whether blockers of glycosylation or phosphorylation prevent the appearance of Ca^{2+} -sensitivity.

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Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain

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To date no nucleic acid has been found in the purified infectious agent which causes the spongiform encephalopathy known as scrapie. In an attempt to identify a unique scrapie virus-associated messenger RNA in tissues of infected animals, we have synthesized an oligonucleotide probe complementary to the mRNA sequence corresponding to the amino-acid sequence of the prion protein, PrP27-30 (ref. 1). We report here that, with this probe, a complementary DNA clone representing PrP27-30 was obtained from scrapie-infected mouse brain; the DNA sequence of this clone could be translated into a protein that matches exactly the published sequence of PrP27-30. The cDNA clone hybridized to a single 2.4-2.5-kilobase (kb) mRNA from both normal and scrapie-infected brain. Thus, the PrP27-30 mRNA is not uniquely associated with scrapie infectivity, suggesting that PrP27-30 may be a normal component of mouse and hamster brain.

Scrapie is a slowly progressive spongiform encephalopathy of sheep and goats² which can be transmitted to a variety of animal species including mice, hamsters and mink³. The pathology of scrapie is similar to certain devastating dementias of humans such as kuru, Creutzfeldt-Jacob disease and Gerstman-Straussler syndrome⁴⁻⁶. The replicating infectious agent which causes these diseases has many properties similar to those of a virus⁷, but has not shown sufficient biophysical homogeneity to allow adequate purification for precise biochemical analysis^{8,9}. Thus, the presence of a viral nucleic acid has not yet been demonstrated. In fact, in the case of scrapie, it has been proposed that the agent may consist solely of protein, and the term 'prion' has been coined to describe such proteinaceous infectious particles¹⁰⁻¹². Recent studies have suggested that scrapie infectivity is associated with large characteristic fibril structures¹³, termed scrapie-associated fibrils¹⁴ or rods¹⁵, which have a unique morphology when negatively stained and examined under the electron microscope but share some of the properties of amyloid which is a major feature of brain pathology in some scrapie models¹⁶. The proteins in these structures isolated from hamster scrapie have also been analysed serologically and biochemically and consist mainly of a polypeptide of relative molecular mass 27,000-30,000, PrP27-30 (refs 1, 13, 14, 17). The N-terminal 15 amino acids of this protein have been sequenced and show no homology with other known proteins¹. Based on this amino acid sequence, we have synthesized a mixture of oligonucleotides for use as a hybridization probe to analyse mRNA populations derived from infected and uninfected animals and to identify unique scrapie virus-encoded mRNA species.

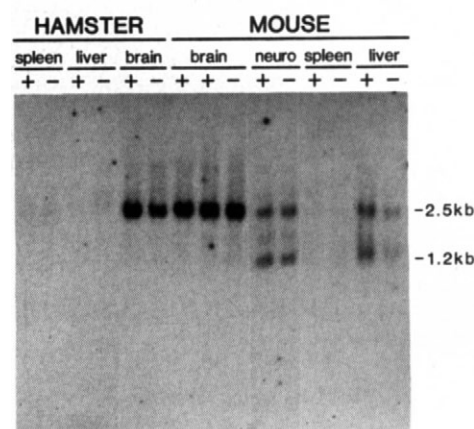


Fig. 1 Identification of scrapie-specific mRNA in infected (+) and uninfected (-) animals. Messenger RNA was isolated from RML outbred mice and Syrian hamsters who showed clinical neurological symptoms 120-150 days (mouse) or 65-75 days (hamster) after intracerebral inoculation of $10^{6.8}$ (mouse) or 10^9 (hamster) infectious units of scrapie. The mouse-adapted Chandler strain of scrapie was obtained from Dr W. J. Hadlow (RML). Hamster-adapted scrapie was obtained from Dr S. Prusiner, University of California School of Medicine, San Francisco. mRNA from uninfected, age-matched animals and from scrapie-infected and uninfected cells of a mouse neuroblastoma cell line (clone NA, obtained from Dr D. Lodmell, RML), was also isolated. Tissue culture cells, brain, spleen and liver tissue were homogenized in 5 vol. of 4 M guanidinium isothiocyanate and disrupted briefly in a blender³⁰. RNA was pelleted by centrifugation through 5.7 M CsCl in 0.1 M EDTA at 35,000 r.p.m. for 16 h at 20 °C in a Beckman SW50.1 rotor. For brains, the RNA pellet was resuspended in 1% SDS, 10 mM Tris-HCl pH 7.4, 5 mM EDTA and extracted with 4:1 chloroform/1-butanol. The aqueous phase was brought to 0.3 M sodium acetate and RNA precipitated with 2.5 vol. ethanol at -20 °C. For liver and spleen, the RNA pellet from the CsCl spin was resuspended in 7.5 M guanidinium-HCl, 20 mM sodium acetate pH 7.0 and 1.0 mM dithiothreitol (DTT) and precipitated with 0.5 vol. ethanol at -20 °C. The pellet was resuspended in water, brought to 0.3 M with sodium acetate and precipitated with 2.5 vol. ethanol at -20 °C. For brain, liver and spleen, RNA pellets were resuspended in water, adjusted to 20 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.5 M LiCl, 0.2% SDS, then passed through an oligo(dT)-cellulose column, washed with similar buffer, and poly(A) RNA was eluted with H₂O. Poly(A) RNA was brought to 0.3 M sodium acetate and precipitated with ethanol at -20 °C as before. RNA was stored at -20 °C as an ethanol precipitate. For analysis of mRNA, 3-6 µg of RNA was pelleted from the ethanol suspension, dried, redissolved in 0.01 M sodium phosphate buffer pH 7.4 containing 2 mM EDTA, 50% formamide and 6% formaldehyde, heated at 68 °C for 5 min and fractionated by electrophoresis in 1% agarose in 0.01 M sodium phosphate pH 7.4, 6% formaldehyde for 16 h at 20 V. Gels were then blotted onto nitrocellulose in 20× SSPE²⁶, and baked at 80 °C in a vacuum for 2 h. For hybridization to the oligonucleotide probe, filters were prehybridized in 6× SSPE, 0.1% SDS, 0.002 M sodium pyrophosphate, 100 µg ml⁻¹ sheared herring sperm DNA and 1× Denhardt's at 37 °C for 3 h. Oligonucleotides were synthesized on a SAM-1 DNA synthesizer (Biosearch, San Rafael, California). To include all possible nucleotide sequences giving the published PrP27-30 amino-acid sequence, 32 different oligonucleotides were synthesized in a mixture as shown in Table 1; 0.1 µg of the oligonucleotide mixtures was labelled with 0.2 mCi of [γ -³²P]ATP in 100 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 10 mM DTT using two units of polynucleotide kinase for 1 h at 37 °C. Non-bound radioactivity was removed by binding the oligonucleotides to a DE52 column in 0.1 M NaCl in TE (10 mM Tris-HCl pH 7.5, 1 mM EDTA), and eluting with 1.0 M NaCl in TE. The prehybridized filter was rinsed in 6× SSPE, 0.1% SDS, 0.002 M sodium pyrophosphate and the ³²P-labelled oligonucleotide was allowed to hybridize to the filter in this buffer at a concentration of 2×10^6 d.p.m. ml⁻¹ at 37 °C for 18 h. Filters were then washed repeatedly in hybridization buffer without probe at 42 °C, and were exposed to film at -70 °C using a Lightning-Plus intensifying screen.

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Table 1 Protein, mRNA and DNA sequences of PrP27-30, oligonucleotide probe and homologous cDNA clone

PrP27-30:																				
Predicted mRNA	5'	Gly	Gln	Gly	Gly	Gly	Thr	His	Asn	Gln	Trp	Asn	Lys	Pro	Ser	Lys				
Oligonucleotide:		GGN	CAR	GGN	GGN	GGN	ACN	CAY	AAY	CAR	UGG	AAY	AAR	CCN	UCN	AAR				3'
cDNA:	5'C	GGA	TGG	GGC	CAA	GGA	GGG	GGT	ACC	CAT	AAT	CAG	TGG	AAC	AAG	CCC	AGC	AAA		
Predicted amino acids:		Gly	Trp	Gly	Gln	Gly	Gly	Gly	Thr	His	Asn ₁₀	Gln	Trp	Asn	Lys	Pro	Ser	Lys		
		CCA	AAA	ACC	AAC	CTC	AAG	CAT	GTG	GCA	GGG	GCT	GCG	GCA	GCT	GGG	GCA			
		Pro	Lys	Thr ₂₀	Asn	Leu	Lys	His	Val	Ala	Gly	Ala	Ala	Ala ₃₀	Ala	Gly	Ala			
		GTA	GTG	GGG	GGC	CTT	GGT	GGC	TAC	ATG	CTG	GGG	AGC	GCC	GTG	AGC	AGG			
		Val	Val	Gly	Gly	Leu	Gly	Gly ₄₀	Tyr	Met	Leu	Gly	Ser	Ala	Val	Ser	Arg			
		CCC	ATG	ATC	CAT	TTT	GGC	AAC	GAC	TGG	GAG	GAC	CGC	TAC	TAC	CGT	GAA			
		Pro ₅₀	Met	Ile	His	Phe	Gly	Asn	Asp	Trp	Glu	Asp ₆₀	Arg	Tyr	Tyr	Arg	Glu			
		AAC	ATG	TAC	CGC	TAC	CCT	AAC	CAA	GTG	TAC	TAC	AGG	CCA	GT	3' 237				
		Asn	Met	Tyr	Arg	Tyr ₇₀	Pro	Asn	Gln	Val	Tyr	Tyr	Arg	Pro ₇₈						

R = A or G; Y = C or T (or U); N = any base. mRNA from scrapie-infected mouse brain was used to synthesize cDNA using the conditions of Okayama and Berg²² with an oligo(dT) primer. Reverse transcriptase was obtained from Seikagaku America, Inc., St Petersburg, Florida. mRNA:cDNA duplexes were extracted with phenol-chloroform, precipitated with ethanol, and the second DNA strand synthesized using the method of Gubler and Hoffman²³. After extraction and precipitation as above, *EcoRI* sites were methylated with *EcoRI* methylase and DNA ends filled-in with the Klenow fragment of DNA polymerase. DNA was extracted and precipitated again and was ligated to *EcoRI* 8-mer linkers (New England BioLabs) using T4 DNA ligase. After 18 h at 12 °C, the reaction tubes were heated to 70 °C for 5 min to inactivate the ligase and linkers were digested for 4 h at 37 °C with *EcoRI* (12 units per µg cDNA). Small DNA fragments were removed by microdialysis for 1 h on Millipore VM 0.05-µm filters against *EcoRI* digestion buffer. *EcoRI* was again added for a second cycle of digestion, followed by microdialysis. DNA was then extracted, ethanol-precipitated and ligated to *EcoRI*-digested calf alkaline phosphatase-digested (Boehringer-Mannheim) λgt 11 DNA²⁴. The ligation mixture was packaged using the Packagene system (Promega-Biotec, Madison, Wisconsin) and used to infect *Escherichia coli* strain Y1088 (ref. 25). Plaques were screened by blotting onto nitrocellulose filters²⁶ and were probed with ³²P-labelled oligonucleotide (see Fig. 1 legend). A positive plaque was picked off and purified by re-plating twice; DNA was isolated, digested with *EcoRI* and two insert fragments of 0.9 and 0.8 kb were found. The oligonucleotide hybridized only to the larger fragment. Both fragments were isolated on 1% low-melting point agarose (Sea Plaque; FMC Corp., Rockland, Maine), purified on Elutip ion-exchange columns (Schleicher & Schuell) and subcloned into M13 (mp18)²⁷ (BRL) and pEMBL8 (ref. 28). Complementary clones containing both the 0.8- and 0.9-kb fragments were identified by the C test²⁷, and the partial DNA sequence was determined using the dideoxy method²⁹. The 0.8-kb fragment, which did not hybridize with the oligonucleotide, had a 60-70-base poly(dT) region at the junction with the M13 vector (sequence not shown), indicating that this DNA fragment was copied from the poly(A)-containing 3' end of the mRNA. The 0.9-kb fragment, which hybridized with the oligonucleotide probe, had no poly(dT) sequence at either end, indicating that this fragment was derived from the 5' end of the mRNA. The preliminary DNA sequence of one end of this fragment is shown. The amino-acid translation of this sequence shows exact homology with the published 15-amino-acid sequence of PrP27-30 (boxed). There is an open reading frame which continues as far as the sequence was read (237 bases from the *EcoRI* site of linkage to the M13 vector).

Messenger RNA populations were obtained from brains, livers and spleens of scrapie-infected mice or hamsters. The oligonucleotides hybridized with 1-3 bands in 9 of 15 samples analysed; however, in no case was there evidence for a scrapie-specific mRNA species (Fig. 1). In each case, uninfected cells or organs showed a band(s) similar to that seen in infected cells. The bands exhibited some 'organ specificity' as both hamster and mouse brain and mouse neuroblastoma cells showed a prominent 2.4-2.5-kb band which was not seen in mouse spleen. There was a weaker reaction with a similar-sized band in mouse and hamster liver. After longer exposure of the autoradiographs, a second band (1.3 kb) was seen in mouse liver and two other bands (2.0 and 1.2 kb) were seen in mouse neuroblastoma cells. However, when the blots were washed at higher temperatures (48 °C), most of these bands disappeared and only the 2.5-kb bands seen in the brain and neuroblastoma cells remained (data not shown).

In the hybridization conditions used, the oligonucleotides may have hybridized to mRNAs having rather poor homology. If a cDNA clone of the message for the PrP27-30 could be isolated, a more precise analysis should be obtained. Therefore, we used the oligonucleotides to screen a cDNA library made from scrapie-infected mouse brain. One clone was isolated, and its partial DNA sequence indicated that it could be translated into an amino-acid sequence identical to PrP27-30 at all 15 positions known (Table 1). Thus, this clone seemed to contain a sequence corresponding to the PrP27-30 protein message. Comparison of the predicted amino-acid sequence shown in Table 1 with a computer bank of known sequences has revealed to date no close homology with any protein tested, except

PrP27-30. When the clone was used as a probe against various mRNAs, a prominent 2.4-2.5-kb band was seen in both normal and scrapie-infected mouse and hamster brain (Fig. 2); there was no evidence for any unique mRNA associated with scrapie infection. Furthermore, there was no reactivity with any mRNA in mouse spleen, which is known to contain significant infectivity¹⁸⁻²⁰. Liver mRNA also failed to hybridize with the cDNA clone, indicating that PrP27-30 was not synthesized in this organ and reactivity of liver mRNA with the oligonucleotide probably detected a message unrelated to PrP27-30.

These results suggest that the published PrP27-30 sequence may not be specific for the infectious scrapie agent; our data imply that a similar protein exists in uninfected mouse and hamster brain. However, if expression of this protein is associated with scrapie disease¹², post-transcriptional regulation could conceivably result in increased levels of the protein in infected brain tissues. It is also possible that the messages detected in normal and infected tissues encode proteins which differ by point mutations or post-translational modifications; this might result in important biological variations similar to those encountered with certain cellular *onc* genes²¹. If this were the case, the normal homologue of the scrapie gene sequence would be endogenous in normal brains. It is interesting to note that PrP27-30 of mouse and hamster must be highly homologous as the hamster PrP27-30 protein sequence was identical to the translated sequence derived from mouse brain cDNA.

Another, to us more likely, interpretation of these results is that PrP27-30 is a normal protein of both mouse and hamster brain which is apparently synthesized in neurones such as the neuroblastoma cell line tested. This protein may be a component

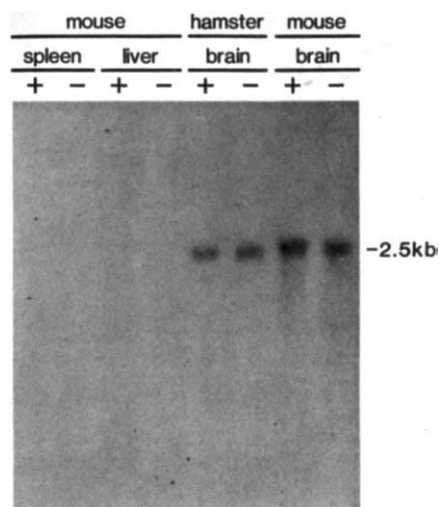


Fig. 2 Hybridization of the PrP27-30 cDNA clone with various mRNAs from hamster and mouse. Poly(A) RNA from various organs was electrophoresed in a formaldehyde agarose gel, blotted onto nitrocellulose, baked for 2 h *in vacuo*, then prehybridized in $2 \times$ SSPE, 0.1% SDS, $5 \times$ Denhardt's, $50 \mu\text{g ml}^{-1}$ denatured salmon sperm DNA at 65°C for 3 h. Hybridization to the nick-translated (specific activity 5×10^7 d.p.m. μg^{-1}) 0.9-kb cDNA clone in the pEMBL8 vector was done in similar conditions for 40 h. Blots were washed three times for 30 min with $2 \times$ SSPE, 0.1% SDS and once with $0.1 \times$ SSPE, 0.1% SDS at 65°C , and were exposed to film. +, Scrapie-infected; -, uninfected. When blots previously hybridized to the oligonucleotides (Fig. 1) were eluted and rehybridized to the cDNA clone, the bands detected migrated in an identical manner to the 2.4–2.5-kb bands detected by the oligonucleotides in mouse and hamster brain and neuroblastoma cell RNA; mouse and hamster liver and spleen RNA on these blots showed no hybridization with the cDNA probe (data not shown).

of the scrapie-associated fibrils which appear to be a consistent pathological feature of scrapie infection and disease; in this case, PrP27-30 would be concentrated during the isolation procedures used to purify the fibrils and the associated scrapie infectivity. It remains to be seen whether this protein has an active or passive role in the pathogenicity of scrapie infection.

We thank Gary Hettrick and Robert Evans for graphics assistance, and Helen Blahnik for preparing the manuscript.

Note added in proof: Since submission of this manuscript we have become aware of results by Oesch *et al.* (*Cell*, in the press) concerning cloning of PrP27-30-specific cDNA from scrapie-infected hamster brain. The DNA sequence of this hamster clone, kindly provided by Dr S. Prusiner, shows 84% base homology with the mouse cDNA sequence given in Table 1.

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Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1

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B-cell stimulatory factor-1 (BSF-1)¹, formerly designated **B-cell growth factor**, is a T-cell-derived factor required for entry into the S phase of the cell cycle by B cells stimulated with low concentrations of anti-IgM antibodies^{2–5}. BSF-1 acts directly on resting B cells to prepare them to synthesize DNA more promptly on subsequent exposure to competent stimuli^{6,7} and to strikingly enhance their expression of class II molecules of the major histocompatibility complex^{8,9}. Previous studies have shown that murine BSF-1 can be separated physically from interleukin-2 (IL-2) and that the molecule has an apparent relative molecular mass (M_r) of ~15,000 and pI values of 6.4–6.7 and 7.4 (ref. 10). Here, we report the production of a monoclonal antibody to BSF-1, its use in characterizing BSF-1, and functional studies demonstrating that this molecule is distinct from IL-1, IL-2 and IL-3.

BSF-1 was obtained from 5–10-l batches of serum-free supernatants of cells of the EL-4 line, a C57BL/6 thymoma, which had been induced with 4 β -phorbol 12 β -myristate 12 α -acetate (10 ng ml⁻¹, Sigma) for 48 h. Partially purified BSF-1 was used to immunize rats, and spleen cells from these animals used to obtain hybridomas (Fig. 1 legend). Subcloning of a selected hybridoma produced two lines, 18F10 and 11B11, both secreting rat IgG1 antibodies, which possessed identical properties and were used interchangeably (Fig. 1 legend).

Figure 1a illustrates the absorption assay for BSF-1 and demonstrates that 18F10 binds a major fraction of BSF-1 activity (9 U) when plates are coated with 40 ng of purified monoclonal antibody (Fig. 1a). Both 18F10 and 11B11 block BSF-1 activity when added directly to co-stimulation assays (Fig. 1b). As little as 8 ng of antibody per 200 μ l culture almost completely inhibited the response of resting B cells to Bet-2, a monoclonal rat anti-mouse IgM¹¹, and 2 U of BSF-1. A rat IgG antibody to

Table 1 Binding to and elution from anti-BSF-1 affinity column

Applied sample	Volume (ml)	BSF-1 activity	
		U ml ⁻¹	Total
(TMS-CpG fraction)	5	5,700	28,500
Effluent	5	49	245
Acetic acid (0.8 M) eluate	0.4	74,000	29,600

A 5-ml sample of TMS-CpG-fractionated EL-4 supernatant was applied to an affinity column (2 ml packed volume). The effluent (5 ml) was collected and the column was washed with 20 ml of NaCl (1 M), 20 ml NP40 (0.5%) in water, 20 ml phosphate-buffered saline and 20 ml water. The column was then eluted with 20 ml of 0.8 M acetic acid. The eluate was lyophilized and reconstituted with 0.4 ml water. BSF-1 activity was measured in the anti-IgM co-stimulation assay using Bet-2.

Fig. 1 Monoclonal anti-BSF-1 absorbs BSF-1 (a) and inhibits BSF-1 activity (b).

Methods. Partial purification of BSF-1, to be described in detail elsewhere (J.O. *et al.*, in preparation), involved adsorption to and elution from trimethyl silyl(TMS)-derivatized controlled pore glass beads (TMS-CpG; Sepharlyte; Analytical Chemicals, Harbor City, California) followed by reverse-phase HPLC. This yielded a >2,000-fold increase in specific activity as measured in units (U) defined by the amount of BSF-1 required for half-maximal responses in the anti-IgM co-stimulation assay, using resting B cells as a test population². Low/Sn rats were immunized with 1.4×10^6 U of TMS-CpG-purified BSF-1 emulsified in complete Freund's adjuvant. Animals were boosted twice with 1.6×10^5 U of HPLC-purified BSF-1 in incomplete Freund's adjuvant, and challenged intravenously with 3.6×10^6 U of HPLC-purified BSF-1. Three days later, spleen cells were fused with the P3-NS-1/1-Ag4-1 line of the P3-X63-Ag8 myeloma. Hybridoma supernatants were screened for anti-BSF-1 activity by an absorption technique in which 24-well culture dishes were coated with ion-exchange chromatography-purified rabbit anti-rat immunoglobulin. Coated culture dishes were incubated with hybridoma supernatants for 4 h, washed and then incubated with a standard amount of HPLC-purified BSF-1 for an additional 4 h. BSF-1 activity remaining in solution was assessed by culturing samples with resting splenic B cells from BALB/c mice in the presence of purified goat anti-mouse IgM ($5 \mu\text{g ml}^{-1}$) or Bet-2. Uptake of ^3H -thymidine was measured after 3 days of culture. Of 72 fusion wells with growing hybridomas, 6 were selected for further study on the basis of this assay. Of these, cells from one fusion well which had reproducible absorptive activity at a high dilution were used in the remaining experiments. The cells from this well were subjected to a series of subclonings, the last of which yielded 18F10 and 11B11, both secreting rat IgG1 antibodies. *a*, Absorption of BSF-1 activity. Tissue culture wells (Cluster 24, Costar) were incubated with $10 \mu\text{g}$ of purified rabbit anti-rat immunoglobulin, washed and incubated with various concentrations of purified 18F10 (anti-BSF-1) or with various dilutions of ascitic fluid containing 3C7 (anti-IL-2 receptor). After washing, HPLC-purified BSF-1 (9 U) was added to wells, allowed to incubate for 4 h and supernatants were tested for residual BSF-1 activity in the anti-IgM (α - μ) co-stimulator assay. Bet-2, a monoclonal α - μ , was incubated with purified B cells (1×10^5 per 0.2 ml) in the presence of absorbed supernatants and uptake of ^3H -thymidine measured 3 days later. As controls, ^3H -thymidine uptake with medium alone, α - μ , BSF-1, or α - μ + BSF-1 are shown. *b*, Inhibition of BSF-1 activity. Purified B cells were incubated with Bet-2 and BSF-1 (2 U) in the presence of various amounts of purified 18F10 or 11B11 (anti-BSF-1) or of various dilutions of ascitic fluid containing 3C7 (anti-IL-2 receptor). Uptake of ^3H -thymidine was measured 3 days later.

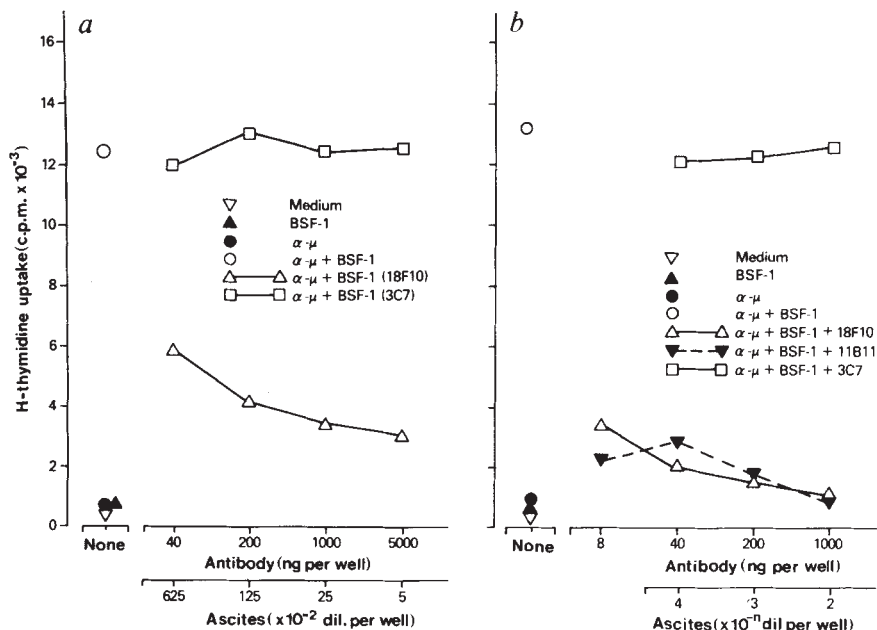


Table 2 Anti-BSF-1 does not block responses to IL-1, IL-2 or IL-3

Response of B cells to anti-IgM and BSF-1					
Stimulant	0	11B11	10 ⁻⁴	10 ⁻³	3C7
None	425	497	403	296	176
α IgM + BSF-1	21,020	1,295	3,425	25,160	18,640
Response of thymocytes to PHA and IL-1*					
	0	18F10	3C7	50C1	
None	660	580	441	336	
PHA ($2 \mu\text{g ml}^{-1}$)	1,395	1,651	1,120	1,313	
PHA + IL-1 (50 U)	90,943	87,877	19,451	68,788	
Response of HT-2 cells to IL-2*					
	0	18F10	3C7	50C1	
None	143	—	—	—	
IL-2 (0.3 U ml ⁻¹)	22,913	19,290	5,300	23,983	
Response of DA-1 cells to IL-3*					
	0	11B11	50C1		
None	40	—	—		
WEHI-3 supernatant (1:5)	22,073	29,590	21,686		

³H-thymidine uptake in response to anti-IgM and BSF-1 (10 U ml^{-1}) was measured at 3 days of culture and responses to IL-1, IL-2 and IL-3 were measured at 2 days of culture and recorded here as c.p.m. Assays were performed as described in ref. 14 for IL-1, ref. 15 for IL-2 and ref. 16 for IL-3, except that in the last case the concentration of the fetal calf serum (FCS) was 2% rather than 10%. 18F10 and 11B11 are rat monoclonal antibodies to BSF-1; 3C7 is a rat monoclonal antibody to the IL-2 receptor; 50C1 is a rat monoclonal anti-dinitrophenyl antibody; 10^{-3} and 10^{-4} refer to dilutions of ascitic fluid. 3C7 inhibits the proliferation of thymocytes in response to phytohaemagglutinin (PHA) and IL-1 because of the involvement of IL-2 in that response; it also inhibits the proliferation of HT-2 cells in response to IL-2, as the IL-2 receptor is critical to such proliferation.

* Ascitic fluid dilution of 10^{-2} used in these experiments.

the IL-2 receptor (3C7, generously provided by Drs G. Ortega, E. Shevach and T. Malek¹²) had no inhibitory activity, indicating that the response of resting B cells to anti-IgM and BSF-1 does not depend on IL-2, and providing a control for nonspecific inhibition.

To characterize BSF-1 more fully, 1.2×10^5 U HPLC-purified BSF-1 was labelled with ¹²⁵I by the chloramine-T method. A sample of this material was incubated with affinity-purified monoclonal antibody and immunoprecipitated with Sepharose beads to which rabbit anti-rat immunoglobulin had been coupled. SDS-polyacrylamide gel electrophoresis (PAGE) of dissolved immunoprecipitates yielded two major bands which migrated with apparent molecular sizes of 19,000–20,000 and 14,000, respectively (Fig. 2a). Two-dimensional gel electrophoresis (isoelectric focusing followed by SDS-PAGE)¹³ indicated that the 19,000–20,000-*M_r* species had a *pI* of 6.7; the material of lower *M_r* was not detected on the two-dimensional gel (Fig. 2b). The *pH* range examined was 4.6–7.1 and it is possible that the 14,000-*M_r* molecule had a *pI* outside this range and migrated off the gel.

HPLC-purified BSF-1 was subjected to SDS-PAGE, then individual lanes were transferred to nitrocellulose paper for protein blotting¹⁴ or cut into fragments for extraction and assay of BSF-1 activity (Fig. 3). ¹²⁵I-labelled antibody bound to two bands, migrating with mobilities of 19,000–20,000 and 14,000, respectively. Analysis of extracted material showed that the bulk of the BSF-1 activity co-migrated with the 19,000–20,000-*M_r* species.

The antibody could also be used for purification of BSF-1 from complex mixtures (Table 1). An affinity column was prepared by conjugating a mixture of 18F10 and 11B11 to CnBr-activated Sepharose 4B (1–2 mg antibody per g Sepharose). TMS-CpG-purified EL-4 supernatant (2.85×10^4 U BSF-1) was applied to a 2.0-ml column; 200 U appeared in the effluent and

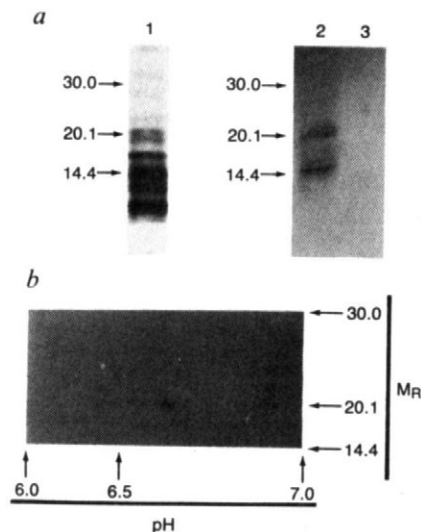


Fig. 2 SDS-PAGE (a) and two-dimensional gel electrophoresis (b) of immunoprecipitates of HPLC-purified ^{125}I -labelled BSF-1. a, SDS-PAGE of HPLC-purified ^{125}I -BSF-1 and of immunoprecipitates prepared with anti-BSF-1 or with normal rat serum. Samples were electrophoresed in 20% polyacrylamide at a constant voltage of 150 V. Autoradiographs were prepared using an intensifying screen and Kodak X-Omat film. Lane 1, SDS-PAGE of HPLC-purified ^{125}I -BSF-1. Lane 2, SDS-PAGE of a sample prepared by overnight incubation of HPLC-purified ^{125}I -BSF-1 with affinity-purified 18F10 and 11B11 (100 μg), absorption with Sepharose 4B, and then binding to rabbit anti-rat immunoglobulin coupled to Sepharose 4B. The sample was dissolved by boiling in 0.1% SDS in 2 mM dithiothreitol, 1 mM EDTA. Lane 3, SDS-PAGE of a sample prepared identically to that in lane 2 except that normal rat serum (10 μl) was used in place of 18F10 and 11B11. b, Two-dimensional electrophoresis of a replicate sample of the immunoprecipitate applied to lane 2 of the SDS-PAGE in a. The dissolved sample was placed in a buffer containing 9.5 M urea, 2% NP40, 2% ampholines (1.6%, pH 5-7; 0.4%, pH 3-10), and 5% β -mercaptoethanol in water and applied to an electrofocusing gel. After focusing, the sample was subjected to SDS-PAGE. The pH range analysed was 4.6-7.1. Only a portion of the autoradiograph is illustrated here. M_r values given are $\times 10^{-3}$.

elution with 0.8 M acetic acid yielded 2.96×10^4 U. When BSF-1 was applied to a control column, prepared by conjugating normal rat immunoglobulin to Sepharose 4B, essentially all applied BSF-1 activity appeared in the effluent.

The antibody has also been used to test the relationship of BSF-1 to several other well-defined lymphokines. Table 2 indicates that although 18F10 ascites at a dilution of 10^{-4} causes marked inhibition of BSF-1 activity, the activities of IL-1, IL-2 and IL-3 tested, respectively, in a thymocyte assay¹⁵, on the growth of HT-2 cells¹⁶ and in the stimulation of an IL-3-dependent T-cell line, DA-1 (ref. 17), are not inhibited even at dilutions of ascitic fluid of 10^{-2} .

We found a discrepancy in the apparent size of the active BSF-1. Previous studies, based on separation of EL-4 supernatants by phenyl Sepharose chromatography and characterization on SDS-PAGE, had indicated respective M_r s for BSF-1 of 15,000-16,000 and 11,000 (ref. 10). The present results indicate that two major molecular species bear an antigenic determinant that reacts with the monoclonal anti-BSF-1 antibody. The larger, 19,000-20,000- M_r species, has BSF-1 activity, whereas the activity of the smaller species remains unresolved. The BSF-1 used in the present studies was prepared from the same cell line used in the previous work, the antibody seems to bind to virtually all material possessing BSF-1 activity in EL-4 supernatant, and the co-stimulation assay used is essentially identical. Gel filtration studies of a human BSF-1-like molecule have revealed a M_r of 17,000 which fell to 14,000 on treatment with 4 M urea³, raising the possibility that changes in apparent

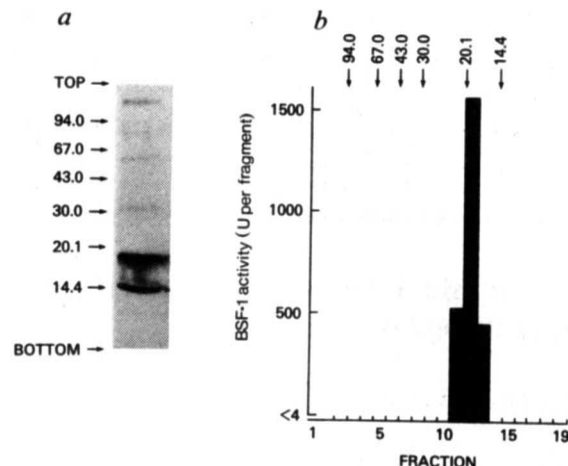


Fig. 3 Protein blotting (a) and BSF-1 activity of HPLC-purified BSF-1 separated on SDS-PAGE (b). a, 2.8×10^4 U of HPLC-purified BSF-1 in 20 μl was separated by SDS-PAGE as in Fig. 2a. Low- M_r markers ($\times 10^{-3}$; Pharmacia) were included in these lanes and in parallel lanes. The contents of this lane were transferred to nitrocellulose paper, using a Transblot Cell (Bio-Rad) and developed with ^{125}I -labelled 11B11. b, 2.8×10^4 U of HPLC-purified BSF-1 in 20 μl was separated by SDS-PAGE. The lane was cut into 0.5-cm fragments which were disrupted in 20% FCS-containing medium by cycles of freezing and thawing; extracted material was assayed for BSF-1 activity and is shown in units per fragment. Low- M_r markers were included in the separated sample and in adjacent lanes; the latter were stained with Coomassie blue to determine the location of each marker.

M_r result from the nature of the purification procedure used. Nonetheless, the relationship between the active 19,000-20,000- M_r species and the 14,000- M_r species remains to be established.

Results indicating that BSF-1 is distinct from IL-1, IL-2 and IL-3 confirm earlier but less direct work^{2-5,10}. This, together with the absence of interferon activity in BSF-1 preparations, indicates that BSF-1 is unique.

Thus, our results demonstrate the value of the monoclonal antibody to BSF-1 as a tool for the molecular characterization and purification of this lymphokine. Indeed, it had previously been proposed that this stimulant be designated BSF-p1, the 'p' reflecting the provisional assignment of the designation in view of the still-partial characterization of the molecule¹. The development of a monoclonal antibody and our more precise characterization now allows us to refer to this as BSF-1.

The finding that the monoclonal antibody is a powerful inhibitor of BSF-1 activity in the anti-IgM co-stimulation assay may allow the role of BSF-1 to be examined in a wide variety of *in vitro* and *in vivo* immune responses, by incorporating the antibody in culture or by treating animals with anti-BSF-1. Such studies are now in progress.

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Anti-idiotypic T cells suppress rejection of renal allografts in rats

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Kidney allografts between inbred rats differing at the major histocompatibility complex (MHC) are normally rejected, usually within 10 to 12 days. In many strain combinations, however, permanent graft acceptance can be induced by either immunological enhancement^{1,2} or a short course of immunosuppressive chemotherapy³⁻⁵. In both cases, prolonged graft survival is accompanied by the appearance in the spleen of a population of suppressor cells⁶. When transferred to a syngeneic host, these cells abrogate or strikingly diminish the rejection response elicited by a renal allograft of the same genotype as the original kidney donor. We have now examined the properties of these suppressor cells and have detected a subpopulation that proliferates *in vitro* when stimulated by irradiated syngeneic T blasts reactive to MHC alloantigens of the kidney donor strain. Comparable proliferation, however, is not induced either by syngeneic blasts reactive to a third strain or by polyclonal syngeneic blasts. These results support the hypothesis that this subpopulation is anti-idiotypic, with specificity for the idiotypes carried by syngeneic T cells stimulated by the kidney allograft. Such anti-idiotypic cells could function as suppressors.

Long-lived immunologically enhanced kidney allografts were induced as follows: AS (RT1¹) recipient rats were given 50×10^6 donor strain spleen cells intravenously 12 days before transplantation, together with three injections of AS anti-donor antiserum on days -11, 0 and +1. This procedure induces donor-specific hyporesponsiveness in the AS recipient, enabling it to accept a semi-allogeneic (AS $\times$ AUG)F₁ kidney graft, and subsequently a fully allogeneic homozygous AUG graft that survives and functions indefinitely. At least two mechanisms seem to be involved in long-term allograft survival. First, the strong immunogenicity characteristic of a primary MHC-incompatible graft is reduced as donor strain dendritic cells are lost from the grafted tissue⁷ and second, the graft recipient progressively develops increasing hyporesponsiveness to the donor genotype, which can be adoptively transferred in a population of splenic T lymphocytes⁸.

Long-term kidney allograft survival can also be induced with the use of immunosuppressive drugs, of which cyclosporin is one of the most effective currently available³⁻⁵. In the rabbit, and in many rat strain combinations, indefinite graft acceptance can be achieved following a limited course of drug treatment for 2-4 weeks^{4,6,9}. Recent evidence suggests that long-term graft survival after pharmacological immunosuppression may depend on similar mechanisms to those responsible for the maintenance stage of immunological enhancement⁶. Similar mechanisms may also apply in clinical organ transplantation, because patients show diminishing requirements for immunosuppression during the later post-transplant period. Consequently, the properties of such putative suppressor cells, in particular their specificity, are of considerable interest.

The experimental protocol to investigate cell specificity *in vitro* is shown in Fig. 1. Strain AS (RT1¹) rats carrying accepted

Strain AS (RT1¹) rat carrying accepted semi-allogeneic (AS $\times$ AUG)F₁ or fully allogeneic AUG (RT1¹) or WAG (RT1^u) kidney graft for at least 100 days

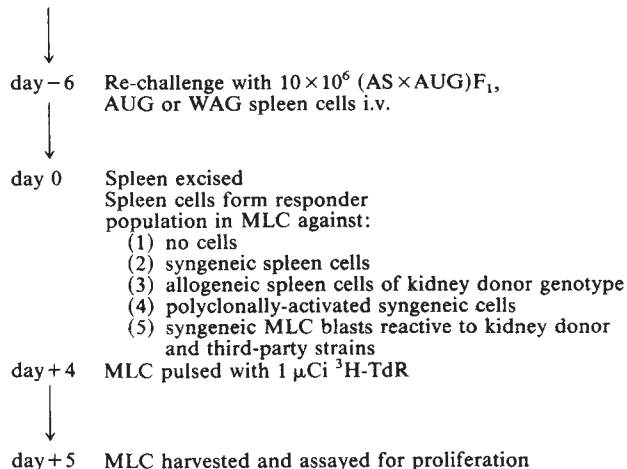


Fig. 1 Model of *in vitro* culture system.

(AS $\times$ AUG)F₁ or AUG (RT1¹) kidneys (immunological enhancement), or AUG or WAG (RT1^u) kidneys (pharmacological immunosuppression) were boosted with 10×10^6 spleen cells of kidney donor genotype on day -6 to maximize the number of suppressor cells present in the spleen. On day 0, the spleens of these animals were excised, and cell suspensions prepared in culture medium (RPMI 1640 supplemented with 2mM L-glutamine, 100 units penicillin and streptomycin per ml, 5% heat-inactivated fetal calf serum v/v). These spleen cells, at 2×10^6 viable lymphocytes per ml, formed the responder population for mixed leukocyte cultures.

Stimulator cells were prepared as follows. Activated blasts of the same strain as the graft recipient (AS, RT1¹) were generated *in vitro* by polyclonal activation or in mixed leukocyte culture (MLC). Polyclonal blasts were collected 3 days after stimulation with concanavalin A (Con A, $1 \mu\text{g ml}^{-1}$), whereas blasts generated in MLC against either the kidney donor strain (AUG or WAG) or against an unrelated third party strain were restimulated (2⁰ MLC) with the same antigen on day 10 of culture, and were harvested 3 days later. Normal spleen cells of both recipient and donor strains were also prepared. Activated blasts and normal cell suspensions were X-irradiated (3,000 rad) before use.

Responder (R) and stimulator (S) populations were then mixed at R:S of 1:1 in the presence of 2-mercaptoethanol (2-ME, 5×10^{-5} M), and were maintained *in vitro* for 5 days (optimal time-course determined from previous experiments, data not shown). The cells were pulsed with $^3\text{H-TdR}$ $1 \mu\text{Ci ml}^{-1}$ for the final 18 h of culture.

Results of cell proliferation in response to the different stimulator populations are presented in Tables 1 and 2. Essentially similar results were obtained using spleen cells from animals with long-surviving kidney allografts irrespective of the method used to ensure protection of the graft. Table 1 presents spleen cell responses of rats with enhanced grafts and Table 2 those of rats that had received immunosuppression consisting of cyclosporin (10 mg per kg per day by gavage for 14 days post-transplant). In each case, spleen cells from animals with long-surviving kidney allografts showed a diminished response to stimulator cells of kidney donor genotype when compared to spleen cells from naive control animals. However, they demonstrated marked proliferation in response to syngeneic 2⁰ MLC blasts reactive to the kidney donor strain. Significantly, this proliferative response was not elicited by polyclonal syngeneic blasts, or by 2⁰ MLC blasts reactive to a third party strain, indicating that cells from the kidney graft recipient were not

Table 1 Proliferative responses of spleen cells from individual AS (RT1^l) rats carrying long-surviving immunologically enhanced (AS×AUG)F₁ or homozygous AUG (RT1^c) kidneys

Source of responder cells	Expt	No cells	Stimulator cells				AS blasts generated in 2° MLC, reactive to:		
			Normal AS spleen	Normal AUG spleen	Con A-stimd AS blasts		AUG	WAG	DA
Strain AS rat carrying (AS×AUG)F ₁ kidney	1	2.1±0.1	2.0±0.0	4.5±0.8	3.4±0.3		7.3±1.1	3.5±0.5	—
	2	1.1±0.4	1.2±0.1	3.6±0.2	3.2±0.4		6.4±0.9	3.0±0.1	—
Control, naive AS	1	1.7±0.2	2.0±0.3	9.2±0.4	3.2±0.2		2.6±0.4	3.1±0.1	—
	2	1.3±0.2	1.3±0.1	6.9±0.4	3.1±0.7		2.7±0.4	2.5±0.4	—
Strain AS rat carrying AUG kidney	3	3.7±0.5	3.9±0.6	5.0±0.9	5.4±1.3		14.4±1.1	—	4.6±0.7
	4	2.2±0.3	2.8±0.1	5.3±0.7	4.4±0.3		9.1±0.5	3.9±0.4	3.2±0.1
	5	1.8±0.1	1.8±0.3	4.5±0.3	—		9.0±0.7	—	3.6±0.2
	6	2.4±0.7	2.5±1.0	5.0±0.4	—		12.0±1.8	—	3.9±0.1
Control, naive AS	3	1.7±0.1	2.3±0.3	16.9±2.1	4.3±0.7		3.4±0.7	—	3.7±0.6
	4	2.7±0.2	3.0±0.1	9.9±1.3	3.9±0.6		2.9±0.1	3.4±0.2	3.4±0.4
	5,6	2.0±0.4	2.1±0.2	9.5±0.6	—		3.1±0.3	—	2.4±0.4

Values represent proliferative activity in MLC (c.p.m. ± s.e. × 10⁻³). Responder and stimulator cell preparation as described in the text. Culture volumes = 200 µl. Six replicates per culture.

Table 2 Proliferative responses of spleen cells from individual pharmacologically suppressed AS (RT1^l) rats carrying long-surviving AUG (RT1^c) or WAG (RT1^b) kidneys

Source of responder cells	Expt	No cells	Stimulator cells				AS blasts generated in 2° MLC, reactive to:		
			Normal AS spleen	Normal AUG spleen	Normal WAG spleen	Con A-stimd AS blasts	AUG	WAG	DA
Strain AS rat carrying AUG kidney	7	1.4±0.0	1.8±0.3	5.1±0.3	8.2±0.4	4.3±0.6	9.4±0.9	4.2±0.3	3.8±0.1
	8	0.9±0.1	0.7±0.0	4.3±0.6	7.8±0.3	—	7.6±1.0	3.5±0.2	1.6±0.2
Strain AS rat carrying WAG kidney	7	1.9±0.4	2.3±0.1	8.0±0.7	4.5±0.2	4.2±0.5	4.0±0.8	8.5±1.0	3.3±0.2
	8	0.6±0.1	0.9±0.2	8.5±0.8	4.5±0.4	—	3.3±0.1	7.4±0.9	2.0±0.1
Control, naive AS	7	1.6±0.2	2.0±0.5	8.7±1.1	8.3±0.8	3.8±0.7	4.0±0.4	3.4±0.1	3.1±0.6
	8	0.8±0.2	0.8±0.3	7.4±0.6	6.8±1.1	—	2.9±0.7	3.0±0.4	1.7±0.1

Values represent proliferative activity in MLC (c.p.m. ± s.e. × 10⁻³). Responder and stimulator cell preparation as described in the text. Culture volumes were 200 µl, with six replicates per culture.

merely being nonspecifically stimulated by activated syngeneic cells. The slight increase in ³H-TdR uptake by responder cells in the presence of these non-specific stimulators may possibly be attributed to secretion of interleukins by activated stimulator cells.

These data suggest that a population of T lymphocytes contained within the spleens of rats bearing accepted renal allografts can specifically recognize and respond to the idiotype determinants present on syngeneic cells reactive to the kidney donor genotype. Other explanations, for example a response against retroviruses carried by syngeneic blasts, are unlikely in view of the specificity of the response. Likewise the notion that the spleen cells from AS rats with long-surviving AUG kidneys proliferate in response to highly immunogenic alloantigen presented by or adsorbed to the surface of the AS anti AUG blasts seems improbable in view of the negligible response of naive AS spleen cells to such blasts. Further, there is a marked diminution in responsiveness of spleen cells from the 'suppressed' AS rats when cultured with AUG stimulator cells. Although not proven at the clonal level, this anti-idiotype subpopulation is a strong candidate for the cell which mediates suppression of kidney allograft rejection *in vivo*.

Suppression of skin-graft rejection induced by autoimmunization with MLC blasts and attributed to anti-idiotype responses was reported by Andersson *et al.*¹⁰; however, it has proved difficult to confirm their experiments, which were based on a concept of shared idiotypes between T and B lymphocytes—an idea still unproven in the context of responses to MHC alloantigens. Dorsch and Roser¹¹, Stockinger¹², and Stephenson and Roser¹³ have presented evidence that neonatally induced toler-

ance is mediated by suppressor cells with anti-idiotypic specificity, and Kimura and Wilson¹⁴ have similarly concluded that resistance to graft-versus-host disease induced in F₁ rats injected with sublethal numbers of parental lymphocytes is due to anti-T cell idotype responses. Our experiments show that a population of cells capable of transferring suppression powerful enough to protect a homozygous MHC incompatible kidney graft from rejection also contains a subpopulation of T cells which have anti-idiotypic specificity *in vitro* for syngeneic clones directed against the MHC antigens of the graft. Taken together, this evidence suggests that anti-T cell idotype responses may underlie many states of unresponsiveness to MHC incompatible tissues, and it raises the question as to whether the same mechanisms are involved in self tolerance.

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Developmental regulation of a cloned adult β -globin gene in transgenic mice

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At different stages of mammalian development, distinct embryonic, fetal and adult haemoglobins are synthesized in erythroid cells, a process termed haemoglobin switching. The cellular and molecular mechanisms controlling haemoglobin switching have been intensively studied, but remain poorly understood¹. To study the developmental regulation of globin gene expression, we have produced transgenic mice²⁻⁶ in which cloned globin genes are present in erythroid cells throughout development. Recently, we reported that adult mice in several transgenic lines carrying a hybrid mouse/human adult β -globin gene, expressed the gene in a correct tissue-specific manner⁷. This finding raised the question of whether an exogenous globin gene could also be subject to appropriate stage-specific regulation. We report here that the hybrid β -globin gene, like the endogenous adult β -globin genes, is inactive in yolk sac-derived embryonic erythroid cells and is expressed for the first

time in fetal liver erythroid cells. Our results indicate that a stage-specific pattern of expression can be conferred by *cis*-acting regulatory elements closely linked to an adult β -globin gene. They also suggest that the embryonic and adult β -globin genes in the mouse are activated (or repressed) by distinct *trans*-acting regulatory factors present in embryonic, fetal and adult erythroid cells.

The timing of expression of the various β -like globin genes during normal mouse development has been well characterized. The first erythroid cells are produced in the blood islands of the yolk sac between days 7 and 11 of gestation, and are released into the embryonic circulation where they persist until day 15-16 (refs 8, 9). Before day 12, these 'primitive' nucleated erythrocytes synthesize the embryonic β -like globin chains γ and z^{10-12} , encoded by two genes termed γ and $\beta H1$ (refs 13, 14); at around day 12, the primitive erythrocytes begin to produce small quantities of the adult β -globin chains^{15,16} in addition to the γ and z chains. The next site of erythropoiesis is the fetal liver, from which smaller, non-nucleated erythrocytes are released, beginning at ~day 12 and continuing until birth¹⁷⁻¹⁹. In fetal liver erythroid cells, all of the β -like globin chains are encoded by the adult β -globin genes, and the γ and $\beta H1$ genes are inactive⁹⁻¹¹. At about day 16, erythropoiesis begins in the spleen and bone marrow, where it continues after birth⁸. The 'adult' erythroid cells produced in these organs display a pattern of globin gene expression similar to that observed in the fetal liver erythroid cells⁹⁻¹¹; thus, the mouse has no separate 'fetal' globin

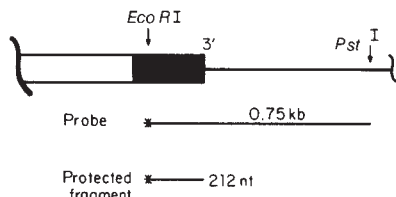
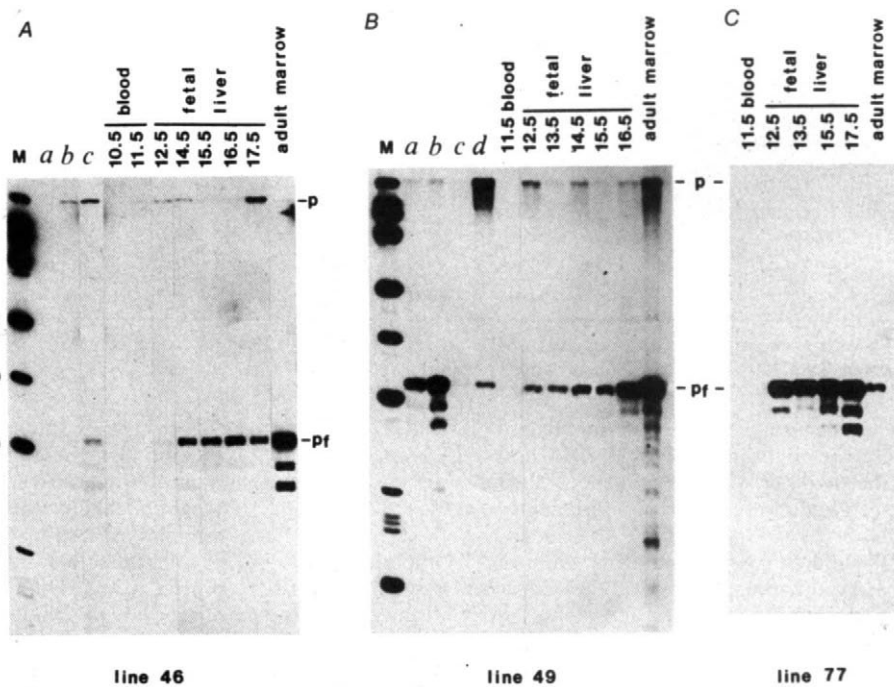


Fig. 1 Analysis of expression of the hybrid mouse/human adult β -globin gene in embryonic, fetal and adult erythroid tissues. Total RNA was isolated from circulating embryonic blood cells and fetal livers, at the indicated days of gestation, and from bone marrow of adult mice. The concentration of hybrid mouse/human β -globin mRNA in each RNA sample (10 μ g) was determined by hybridization with an excess of a probe specific for the 3' (human) end of the mRNA, followed by S_1 nuclease digestion and electrophoresis on a polyacrylamide gel containing 7M urea²²⁻²⁴. p, Position of undigested 0.75-kb probe. pf, Position of the 212-nucleotide protected fragment indicating (see diagram)

correctly terminated human or hybrid mouse/human β -globin mRNA (the bands of lower relative molecular mass (M_r) were not reproducibly observed and are believed to be S_1 nuclease artefacts). It has previously been shown that the hybrid mRNA4 has correct 5' termini¹. M, M_r markers (Φ X174 DNA digested with *Hinf*I). A, Transgenic mouse line 46: lanes a-c, positive control hybridizations with human cord blood RNA samples containing 1.4, 7 and 14 pg, respectively, of β -globin mRNA. B, Transgenic mouse line 49: lanes a-d, human cord blood RNA containing, respectively, 14, 70, 1.4 and 2.8 pg of β -globin mRNA. C, Transgenic mouse line 77. The steady-state level of endogenous β -globin mRNA in both fetal liver and adult bone marrow RNAs, expressed as a fraction of total RNA, is $\sim 10^{-3}$ (Fig. 2 and ref. 7). The approximate steady-state levels of the hybrid β -globin mRNA, determined by comparison with human β -globin mRNA standards, were: line 46 fetal livers (days 14.5-17.5), 3×10^{-6} ; line 46 bone marrow, 1.5×10^{-5} ; line 49 fetal livers (days 12.5-15.5), 5×10^{-7} ; line 49 bone marrow, 10^{-5} ; line 77 fetal livers, 2×10^{-5} ; line 77 bone marrow, 2×10^{-6} . Lines 46 and 49 each contain one copy of the hybrid gene per diploid genome, while line 77 carries three copies⁷.

Methods. Homozygous transgenic males from lines 46 and 49 were mated with normal C57BL/6J females and the resulting hemizygous transgenic embryos were dissected out at various stages of gestation. The day on which the copulation plug was observed was designated day 0. In line 77, which carries the foreign gene on the X chromosome, hemizygous transgenic males were mated with homozygous transgenic females to produce 100% transgenic (hemizygous male or homozygous female) embryos. Embryos in their extraembryonic membranes were first washed in phosphate-buffered saline (PBS) containing 10 U ml⁻¹ heparin (PBS/H) to remove maternal blood. The membranes were then removed, the embryos were bled from the umbilical cord into PBS/H, and the cells were collected by centrifugation. Blood cells or whole fetal livers were pooled from 5-10 embryos at the same stage of gestation, and total RNA was isolated by homogenization in 4M guanidine thiocyanate and centrifugation through CsCl³⁴. RNA concentrations were determined from the A_{260} and confirmed by electrophoresis on denaturing formaldehyde/agarose gels³⁵, which also showed that the RNA preparations were undegraded, as judged by the ribosomal RNA profiles. Preparation of the probe, hybridization and S_1 nuclease analysis were performed as described elsewhere^{6,7}. The concentration of β -globin mRNA in the human cord blood RNA was determined by R_0 analysis (data not shown) to be 1%, in agreement with published estimates of the globin mRNA content of reticulocytes³⁶.



genes. This contrasts with the pattern of haemoglobin switching in humans, in which the adult β -globin gene encodes only ~5% of the β -globin chains in fetal liver erythroid cells (the majority being encoded by the fetal α - γ and γ - γ genes) and becomes maximally active after birth²⁰.

The transgenic mice used in this study carried between one and three copies of a hybrid mouse/human β -globin gene that included the 5' portion of the mouse β^{dmaj} adult globin gene and 1.2 kilobases (kb) of 5' flanking DNA, joined to the 3' portion of the human β -globin gene and 2 kb of 3' flanking human DNA^{7,21}. All of the plasmid vector DNA, except for 350 base pairs, had been removed from the gene before microinjection into mouse zygotes. Adult mice in four different transgenic lines were found to express the foreign globin gene exclusively or predominantly in erythroid tissues⁷. Here we examine the developmental regulation of the hybrid β -globin gene in three of the lines, numbered 46, 49 and 77.

Circulating blood was collected from transgenic embryos at day 10.5 or 11.5 post-coitum (p.c.), a stage at which essentially all of the blood cells are primitive erythrocytes derived from the yolk sac. Whole livers, consisting largely of fetal erythroblasts, were dissected from 12.5–17.5-day p.c. fetuses. In addition, bone marrow was obtained from the femurs of adult transgenic mice. Total RNA was isolated from each of these tissues, and the level of hybrid mouse/human β -globin messenger RNA was measured by hybridization with a ³²P-labelled probe specific for the human portion of the mRNA, followed by digestion with S₁ nuclease, electrophoresis on polyacrylamide gels and autoradiography^{21–24}.

No hybrid mouse/human β -globin mRNA was detected in blood cell RNA from 10.5- or 11.5-day p.c. embryos, in any of the three transgenic lines examined. In contrast, the foreign mRNA was detected in fetal livers at all stages examined, as well as in the adult bone marrow (Fig. 1). For comparison, the expression of the endogenous adult β -globin genes in the same tissues was monitored by hybridization with a probe specific for mouse adult β -globin mRNA (Fig. 2). As expected, the endogenous adult mRNA was detected only in trace amounts in circulating blood of 11.5-day p.c. or earlier embryos, but was present at a high level in the fetal liver and adult marrow. To ensure that the circulating embryonic blood cells analysed were indeed erythroid cells containing globin mRNA, the RNA preparations were hybridized with a probe specific for the mouse embryonic γ -globin mRNA. The γ -globin specific probe hybridized strongly with RNA from 11.5- or 12.5-day p.c. embryonic blood, but not with fetal liver RNAs (Fig. 3). We conclude that the mouse/human hybrid β -globin gene in three different transgenic mouse lines is inactive in embryonic blood cells, and is first expressed in the fetal liver erythroblasts. This pattern of switching is identical to that of the endogenous adult β -globin genes.

Several aspects of the regulation of the exogenous globin gene differed significantly from that of the endogenous adult β -globin genes. First, the steady-state level of the hybrid β -globin mRNA did not exceed 2–3% (per gene copy) of the level of endogenous β -globin mRNA (see Fig. 1 legend) in either fetal liver or adult bone marrow cells⁷. Second, when the relative levels of expression of the foreign gene in fetal and adult erythroid tissues were compared, two different patterns were observed, each somewhat different from that of the endogenous adult β -globin genes. The endogenous adult β -globin mRNA was present at approximately equal levels in fetal liver and adult bone marrow RNA preparations, in each of the transgenic mouse lines (Fig. 2). In mice of lines 46 and 49, the hybrid β -globin mRNA was present at a 5–20-fold lower level in 12.5–15.5-day p.c. fetal liver RNA than in adult bone marrow RNA (Fig. 1). This low fetal/adult expression ratio is characteristic of the adult β -globin gene in humans²⁰, suggesting a possible influence of human β -globin sequences in the hybrid gene. However, in the third transgenic line examined (77), the hybrid β -globin mRNA level was 10-fold higher in fetal liver than in adult bone marrow (Fig. 1), which resembles neither the mouse nor the human pattern.

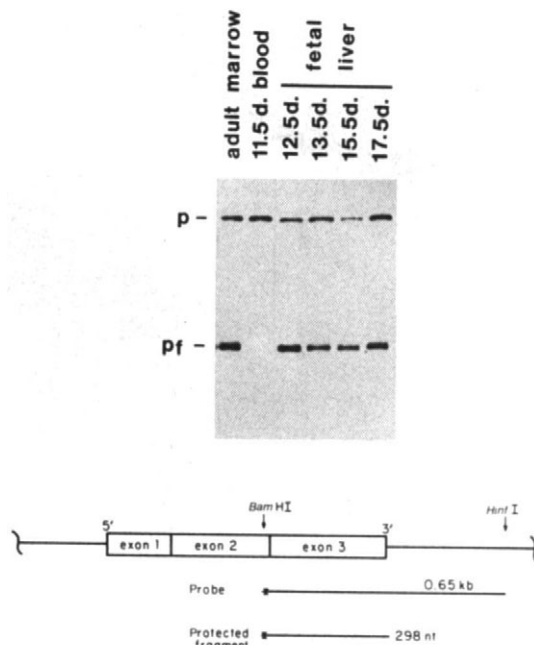


Fig. 2 Analysis of endogenous adult β -globin mRNA in embryonic, fetal and adult erythroid tissues. RNA samples from embryonic blood, fetal liver and adult bone marrow (0.5 μ g of each) from transgenic mice in line 77 (the same RNA preparations as analysed in Fig. 1c) were analysed by hybridization with an excess of a probe specific for mouse adult β -globin mRNA, followed by S₁ nuclease digestion and electrophoresis on a polyacrylamide gel containing 7 M urea. The probe was a *Bam*HI–*Hinf*I fragment derived from a mouse β^{dmaj} globin complementary DNA clone³⁷, and hybridization to either β^{dmaj} globin mRNA or β^s globin mRNA (found in C57BL/6J mice) protects a labelled 298-nucleotide (nt) fragment from S₁ nuclease digestion (see diagram). p, Position of undigested 0.65-kb probe; pf, position of 298-nt protected fragment.

Methods. The *Bam*HI–*Hinf*I fragment of a mouse β^{dmaj} globin cDNA clone was 3' end-labelled with ³²P to a specific activity of 6×10^7 c.p.m. μ g^{–1}, strand-separated on a polyacrylamide gel and eluted²⁴; 2 ng of the probe was incubated with each mouse RNA sample at 45 °C in 80% formamide buffer⁷, then digested with S₁ nuclease^{22,23}. Control hybridizations with varying amounts of mouse blood RNA (data not shown) confirmed that the probe was indeed present in excess over the β -globin mRNA, and that the hybridization signals observed (298-nt protected fragment) were proportional to the concentration of mouse β -globin mRNA in each RNA sample.

Similarly, in a fourth transgenic line, 75A (ref. 7), preliminary data (not shown) indicate that the hybrid gene is expressed at a much higher level in the fetal liver than in adult erythroid tissues. We have recently produced several new transgenic mice, carrying either the human β -globin gene or the cloned murine β^{dmaj} globin gene, and analysis of these lines may help to elucidate the factors influencing the fetal/adult expression ratios of the mouse and human adult β -globin genes.

The main conclusion from the data presented here is that an adult β -globin gene, on a 5-kb DNA fragment, contains the information to remain inactive in primitive erythroid cells which are synthesizing embryonic haemoglobins, and yet to be specifically activated later in development in fetal and adult erythroid cells. This represents the first demonstration that a cloned globin gene can be subject to stage-specific regulation during the transition from embryonic to fetal erythropoiesis. It has already been established that globin genes introduced with limited amounts of flanking DNA can be specifically activated during the *in vitro* differentiation of erythroid cell lines^{21,25–28} and preferentially expressed in erythroid rather than non-erythroid cells both in tissue culture^{29,30} and in transgenic mice⁷. However, the possibility that globin gene switching requires much more DNA

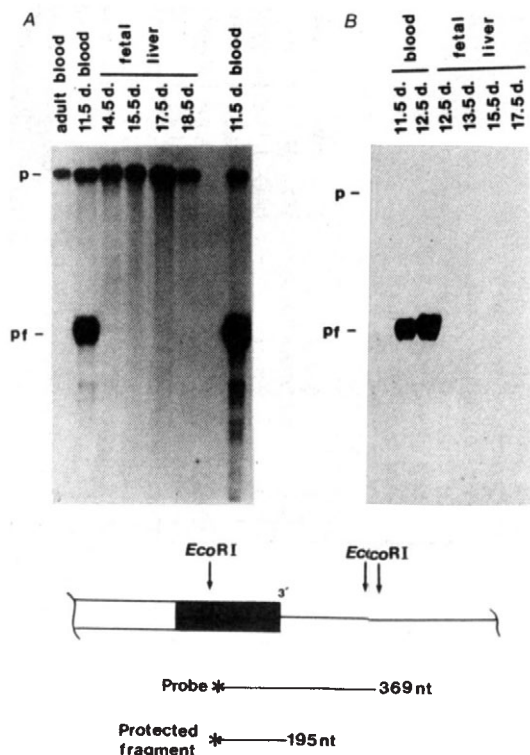


Fig. 3 Detection of endogenous γ -globin mRNA in embryonic blood cells. RNAs from embryonic blood and fetal livers of mice in lines 46 (A) and 77 (B) were analysed for the presence of mRNA from the mouse embryonic γ -globin gene; 0.1 μ g of each RNA sample was hybridized with 3 ng of a 3' end-labelled, strand-separated probe derived from the 3' end of the mouse γ -globin gene¹³. The hybrids were digested with S_1 nuclease and analysed by electrophoresis on a polyacrylamide gel containing 7 M urea. p, Position of undigested probe (369 nt); pf, position of the 195-nt protected fragment. The lane marked 'adult blood' is a control hybridization with whole blood RNA from an adult mouse. The two lanes in A marked '11.5-day blood' show hybridization with two blood cell RNA preparations from different batches of 11.5-day-old embryos.

Methods. A pBR322 plasmid containing the 6-kb *Hind*III mouse DNA fragment including the γ -globin gene¹³ was digested with *Eco*RI, 3' end-labelled with ³²P, and the 369-nt fragment shown in the diagram was purified on a strand-separating polyacrylamide gel. The probe had a specific activity of 5×10^7 c.p.m. μ g⁻¹ and hybridization was performed at 45 °C.

sequence information was suggested by several lines of evidence, such as the observation that deletions several kilobases 3' to the human γ -globin genes can block their normal postnatal inactivation (reviewed in ref. 31).

Our data do not exclude the possibility that more distant sequences in the β -globin cluster have an important role in regulation of the adult β -globin gene. Indeed, their absence from the microinjected globin gene is one of several factors that may be responsible for the low level of expression observed in the transgenic mice, as discussed previously⁷. However, the ability of a single adult β -globin gene, removed from the context of an intact β -globin gene cluster or 'domain'^{32,33}, to be nevertheless switched-on at the appropriate developmental stage, argues that distant sequences in the β -globin domain are not required *in cis* for this particular regulatory event to occur. Our results suggest a model in which the switch from embryonic to adult β -globin in the mouse is mediated by the presence of distinct *trans*-acting factors in embryonic as opposed to fetal and adult erythroid cells, which interact with DNA sequences closely linked to the embryonic and adult genes. Whether these regulatory factors are positive or negative, and the number and location of the DNA sequences with which they may interact, are questions that can be addressed by constructing hybrid genes between

embryonic and adult β -globin genes, or between globin and non-globin genes^{26,27} and examining their developmental regulation in transgenic mice.

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Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11;14) chromosome translocation

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The t(11;14)(q13;q32) chromosome translocation has been reported in diffuse small and large cell lymphomas and in chronic lymphocytic leukaemia (B-CLL)^{1,2} and multiple myeloma³. Because chromosome band 14q32 is involved in this translocation, as well as in the t(8;14)(q24;q32) translocation of the Burkitt tumour⁴, interruption of the immunoglobulin heavy-chain locus was postulated for this rearrangement^{5,6}. We have cloned the chromosomal joinings between chromosomes 11 and 14 and also

between chromosomes 14 and 18, in B-cell tumours carrying translocations involving these chromosomes^{6,7}, and suggested the existence of two translocated loci, *bcl-1* and *bcl-2*, normally located on chromosomes 11 (band q13) and 18 (band q21) respectively, involved in the pathogenesis of human B-cell neoplasms^{6,7}. The results indicate that in the leukaemic cells from two different cases of CLL, the breakpoints on chromosome 11 are within 8 nucleotides of each other and on chromosome 14 involve the *J4*-DNA segment. Because we detected a 7mer-9mer signal-like sequence with a 12-base-long spacer on the normal chromosome 11, close to the breakpoint, we speculate that the t(11;14) chromosome translocation in CLL may be sequence specific and may involve the recombination system for immunoglobulin gene segment (*V-D-J*) joining.

Our previous analysis of the breakpoint between chromosomes 11 and 14 in one case of B-CLL (CLL 271) indicated that it occurred in the joining (*J*) region⁶ of the heavy-chain locus⁵ on chromosome 14. Chromosome 11-derived DNA sequences close to the breakpoint in the CLL 271 cells were then used to detect rearrangements of the homologous DNA sequences in neoplastic cells of a patient with a diffuse B-cell lymphoma with the t(11;14) chromosome translocation (LN87) (ref. 6). These initial results indicated that the chromosomal breakpoints in these two cases were close to each other. In the present study we have examined the structure of the chromosome breakpoint in CLL 271 cells and have compared it with another case of B-CLL (CLL 1386).

Using the chromosome 11-derived DNA probe near the chromosomal breakpoint on the 14q⁺ chromosome from CLL 271 (Fig. 1; ref. 6), we screened DNAs from several human B-cell neoplasms for rearrangements of the homologous DNA sequences and detected rearrangement in sample CLL 1386. Frozen CLL 1386 cells stored in liquid nitrogen were defrosted, stimulated to divide with B-cell mitogens⁸ and karyotyped. CLL 1386 cells were found to have the characteristic t(11;14) (q13;q32) translocation (Fig. 2).

Southern blots of *Bcl*I, *Hind*III and *Sst*I digested DNAs derived from CLL 271, CLL 1386 and LN87 were then hybridized with probes *a* or *b* (see Fig. 1B). We detected germline restriction fragment(s) in normal human DNA, whereas an additional fragment, depending on the restriction enzyme and probe used, was found in CLL 271, CLL 1386 and LN87 DNA (Fig. 1A).

The results shown in Fig. 1A indicate that the breakpoint in CLL 271 is between the *Sst*I¹ and *Hind*III² sites and the breakpoint of LN87 is between the *Hind*III² and the *Sst*I² sites. Digestion of DNA from another case (CLL 1386) with *Sst*I did not result in the appearance of a rearranged DNA fragment, so the chromosome 11 breakpoint may be mapped between the *Hind*III¹ and the *Sst*I¹ sites. However, CLL 1386 DNA shows only a germline *Hind*III¹/*Sst*I¹ fragment (data not shown). Because the breakpoint of CLL 1386 is mapped close to the *Sst*I¹ site in the *Sst*I¹/*Hind*III² fragment, the result of digestion of CLL 1386 DNA with *Sst*I (shown in Fig. 1A) may reflect co-migration of a rearranged fragment with a germline DNA fragment.

We attempted to clone the joining between chromosomes 11 and 14 on the 14q⁺ chromosome of CLL 1386 cells to compare it with that in CLL 271 cells, which we had already isolated⁶. The DNA of CLL 1386 cells was partially digested with *Sau*3A restriction enzyme and DNA fragments of 15–23 kilobases (kb) were collected by size fractionation using sucrose density gradient centrifugation. These were ligated to the λ phage vector EMBL3A DNA (ref. 9) and packaged *in vitro*. By screening 200,000 recombinant clones with probes *a* and *c* (Fig. 1), we obtained five independent clones. Two of the five clones also hybridized with a probe specific for the heavy-chain locus joining (*J_H*) segments (pHj)⁶, indicating that they contained the joinings between chromosomes 11 and 14 on the 14q⁺ chromosome and also that the breakpoint on chromosome 14 was close to the *J_H* region. The restriction maps of the region surrounding the 11;14 chromosome joinings in CLL 1386 cells and of the corresponding normal chromosome 11 and the breakpoint in CLL 271 cells

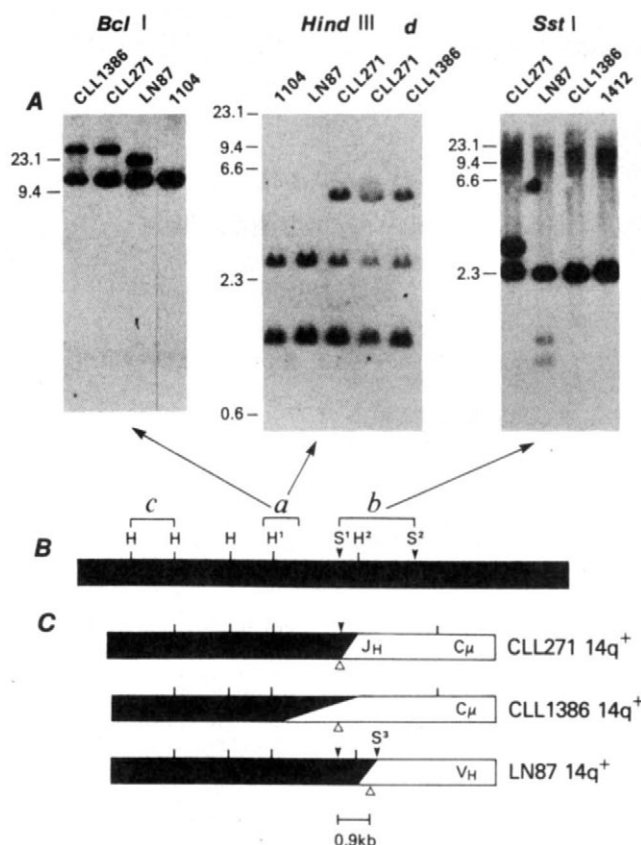


Fig. 1 Southern blot hybridization of CLL 271, CLL 1386 and LN87 DNA with human chromosome 11-derived probes (A) and the maps of chromosome 14q⁺ in CLL 271, CLL 1386 and LN87 (C) and of normal chromosome 11 (B).

Methods. A, DNAs derived from neoplastic cells of CLL 271, CLL 1386 and LN87 were cut with the restriction enzymes shown, run on 0.7% agarose gels and transferred to nitrocellulose filters. The filters were hybridized with nick-translated probe *a* (pRc8SmR)⁶ or *b* (shown in B). Hybridizations were carried out in 50% formamide/4×SSC at 37°C and finally washed with 0.2×SSC at 65°C. DNA 1104 and 1412 were obtained from T-cell lymphomas and used as germline control for chromosome 11. The size is shown in kb beside each panel. B, C, Structure of normal chromosome 11 in B is deduced by analysing the chromosome 11 sequence-containing recombinant clones in Fig. 3 obtained from CLL 271 and CLL 1386 genomic phage libraries. Filled box and open box show chromosomes 11 and 14, respectively. The cleavage sites by restriction enzyme *Sst*I and *Hind*III are shown by S (▲) and H (□), respectively. All restriction sites are not shown. Open triangles represent the joining sites between chromosomes 11 and 14 on chromosome 14q⁺ which are determined as described in the text.

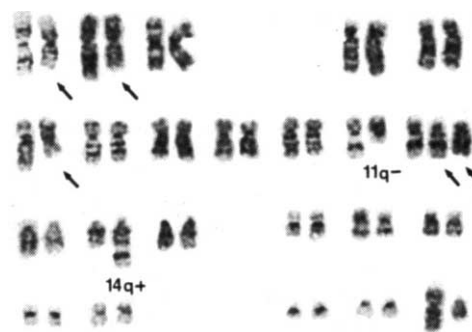


Fig. 2 Representative Giemsa-banded karyotype from CLL 1386 interpreted as: 47, XY, del(1)(p11p21)(q22q25), del(2)(q31), del(6)(q13-15), t(11;14)(q13;q32), inv(12)(p13q13), +inv(12)(p13q13). Abnormalities other than the t(11;14) translocation are indicated by arrows.

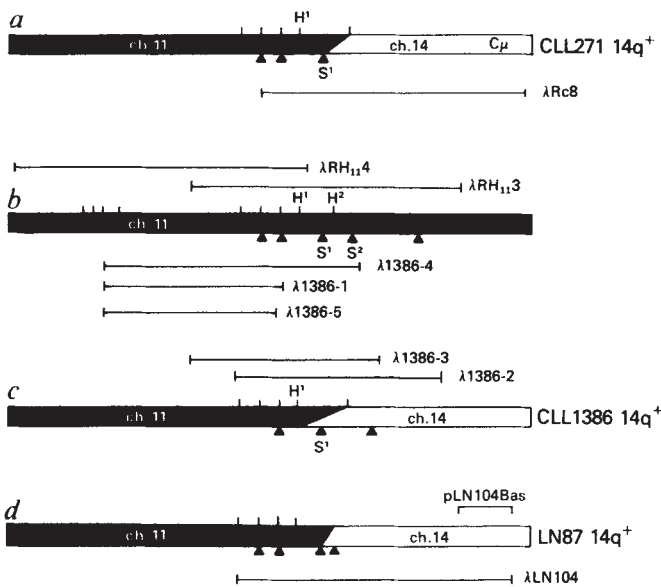


Fig. 3 Restriction maps of the regions surrounding the breakpoints on chromosome 14q⁺ in CLL 271 (a), CLL 1386 (c), LN87 (d) and of normal chromosome 11 (b). Filled and open boxes represent chromosomes 11 and 14, respectively. Horizontal lines represent the insert DNAs of representative recombinant clones. λRc8 has been described previously⁶. λRH11-3 and -4 were obtained by screening the CLL 271 library with chromosome 11 sequences. λ1386s were obtained from the CLL 1386 library as described in the text. λ1386-1, 4 and 5 all contain only DNA sequences of chromosome 11. The cleavage sites of restriction enzyme *Sst*I and *Hind*III are shown by H (I) and S (▲), respectively.

are shown in Fig. 3. As more detailed restriction mapping showed that the chromosome 11 breakpoints in CLL 271 and CLL 1386 are both very close to the *Sst*I¹ site (data not shown), we determined the nucleotide sequences surrounding these breakpoints, and the corresponding segment of normal chromosome 11 from CLL 271 cells. The sequences obtained are shown in Fig. 4, and indicate that the first 31 bases following the common *Sst*I¹ site are identical between the normal chromosome 11 and the 14q⁺ joining region of the two CLLs. The sequences first start to diverge between normal chromosome 11 and the 14q⁺ segment of CLL 1386. Then, just 8 bases further, the DNA sequences of the normal chromosome 11 and that of the 14q⁺ joining region of CLL 271 diverge.

Because the breakpoint on chromosome 14 in CLL 271 cells was mapped in the *J_H* region of the immunoglobulin heavy-chain locus⁶, we expected to find DNA sequences of the *J_H* region beyond the breakpoint. As shown in Fig. 4, beyond the breakpoints of both CLL 271 and CLL 1386 cells, the DNA sequences are almost identical to the *J4* segment of the heavy-chain locus¹⁰, although there are some unidentified sequences between the

breakpoints on chromosome 11 and chromosome 14 of both cases. Addition of a few extraneous nucleotides during variable-diversity-joining (*V-D-J*) segment joining has been described¹¹.

Thus the breakpoints of CLL 271 and CLL 1386 on both chromosome 11 and chromosome 14 are very close to each other. Although the short sequence of 9 bases between the breakpoints on chromosomes 11 and 14 in CLL 271 is of unknown origin, the comparable sequence in CLL 1386 between chromosome 11 and 14 sequences on the 14q⁺ chromosome shows some similarity to the corresponding region on the normal chromosome 11. Therefore, this sequence in CLL 1386 cells seems to have been generated by disturbance of normal chromosome 11 sequences during the translocation event. This may move the breakpoint on chromosome 11 in CLL 1386 slightly closer to chromosome 14 sequences. DNA sequences 3' to *J4* coding sequences are identical in the 14q⁺ chromosomes of CLL 271 and CLL 1386, although these sequences show a considerable number of base changes from the published DNA sequences 3' to the *J4* segment¹⁰.

Note also that the breakpoints on chromosome 14 in both our cases of CLL are in the region where the *D* segment is joined to the *J_H* segment during normal *V-D-J* joining to produce an active immunoglobulin gene. This surprising finding suggests the involvement in the t(11;14) translocation of a recombinase responsible for *V-D-J* joining. This putative recombinase is thought to recognize conserved DNA sequences called '7mer-9mer', located close to *V*, *D* and *J* segments of immunoglobulin genes in germline DNA¹². It has been proposed that the 7mer-9mer sequence with a 12-base-long spacer is paired with a 7mer-9mer sequence with a 23 ± 1-base-long spacer during *V-D-J* joining¹². Interestingly, as shown by brackets in Fig. 4, DNA of normal chromosome 11 has a sequence very similar to this conserved 7mer-9mer signal sequence, very close to the breakpoint. This 7mer-9mer sequence on chromosome 11 is separated by a 12-base-long spacer, whereas the 7mer-9mer sequence of the *J4* segment is separated by a 23-base-long spacer. These two sets of sequences can be drawn into a secondary structure, as shown in Fig. 5. Although the complementarity between the 7mer of chromosome 11 and the *J4* segment is not very striking (3 bases in 7), this complementarity does not seem to be absolutely necessary even for normal *V-D-J* joining. The 7mer sequence on chromosome 11 is perfectly palindromic, which is one of the characteristics found in most 7mer sequences¹². The homology to the consensus 9-mer is much stronger (8 of 9 residues). Thus, the t(11;14) chromosome translocation in two separate cases of CLL seems to be sequence specific, suggesting that the recombination system for *V-D-J* joining is involved, and that the translocation may be mimicking normal *V-D-J* joining. This also suggests that the putative recombinase can function even between two independent chromosomes.

The restriction map of the DNA region encompassing the breakpoint of the t(11;14) translocation in a diffuse B-cell lymphoma (LN87) and of the corresponding normal chromosome 11 are also shown in Fig. 3. The recombinant clone contain-

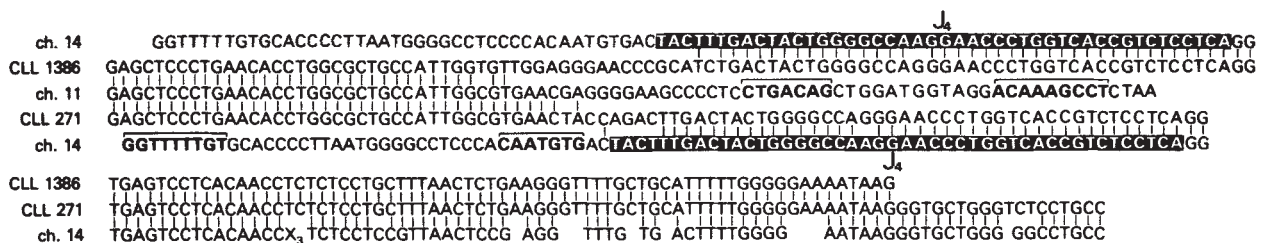


Fig. 4 DNA sequences of the joining sites between chromosomes 11 and 14 in CLL 271, CLL 1386 and of corresponding normal chromosome 11. Identical nucleotide sequences are shown by vertical lines. The boxed region indicates the *J4* coding segment of the immunoglobulin heavy-chain gene¹⁰. The DNA sequences shown by brackets on chromosome 14 indicate the conserved sequence 7mer-9mer (see text). DNA sequences were obtained from the *Sst*I¹ (S¹) site shown in Fig. 3 to the chromosome 14 sequence by using the chemical degradation method of Maxam and Gilbert¹⁴ with a modification of the A reaction¹⁵.

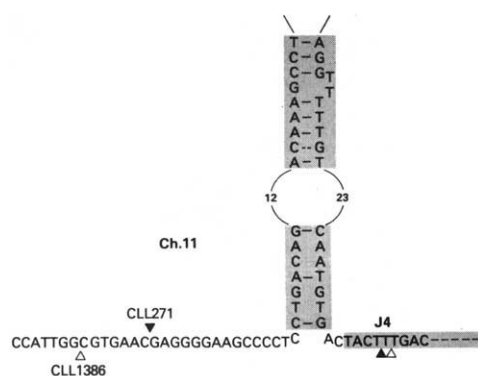


Fig. 5 A possible secondary structure between chromosome 11 and the J4 segment of the immunoglobulin gene.

ing the breakpoint was isolated from an LN87 genomic library as described above. The chromosomal breakpoint in LN87 cells was mapped very close to the *Sst*I² site in Fig. 1B by comparing the detailed restriction maps of normal chromosome 11 clones and a breakpoint-containing clone (data not shown). The chromosome 11 breakpoint in these cells is thus ~0.9 kb away from the site in the two CLLs. In addition, the breakpoint on chromosome 14 in LN87 cells seems to be quite different from that of CLL 271 and CLL 1386. It seems to be either 5' to or within an immunoglobulin V-region gene, because a DNA probe, pLN104Bas (see Fig. 3), hybridized with multiple restriction fragments of human genomic DNA (data not shown). Such a pattern is characteristic of immunoglobulin heavy-chain V-region sequences¹³.

Thus the chromosome breakpoints in the B-cell lymphoma LN87 are different, on both chromosomes 11 and 14, from those in the two CLLs we examined. It is possible that in the case of LN87, a different mechanism may have contributed to the chromosome translocation. Sequence analysis of the joining between chromosomes 11 and 14 on the 14q⁺ chromosome of LN87 cells should provide useful information concerning this issue.

These results also indicate that the breakpoints on chromosome 11 in three cases of B-cell neoplasia with the t(11;14) translocation, two CLLs and one B-cell lymphoma are all sufficiently close as to be detected by genomic Southern blot hybridization using a single restriction enzyme such as *Bcl*I and a single DNA probe such as *a* in Fig. 1B. This may make it possible to take practical advantage of specific DNA probes for the diagnosis of B-cell leukaemias and lymphomas carrying the t(11;14) translocation.

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In vivo somatic mutations in human lymphocytes frequently result from major gene alterations

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Somatic mutations, either spontaneous or produced by identifiable mutagens, are thought to be important in the aetiology of cancer and in the ageing process. The study of somatic mutations in human cells *in vivo* has recently been made possible by the development of techniques for enumeration and clonal expansion of lymphocytes mutated at the chromosome X-linked hypoxanthine phosphoribosyl transferase (HPRT) locus^{1,2}. We have studied the molecular basis of *in vivo* *hprt* mutations in human lymphocytes and report here that a surprisingly high proportion (57%) involve substantial gene alterations which are not evident cytogenetically. These major gene alterations include deletions, exon amplifications and novel, sometimes amplified, bands on Southern analysis. Such changes emphasize the fluid nature of information in DNA and may be indicative of general mechanisms by which functional gene loss is involved in the aetiology of cancer and the homeostatic failure of ageing.

Mutant HPRT⁻ cells can be selected for by the purine analogue 6-thioguanine (TG) as described previously^{1,3}. Lymphocytes obtained from the blood of healthy adult males were cloned at limiting dilution either in the absence of TG (unselected, presumably HPRT⁺ clones) or in the presence of 2.5 µg ml⁻¹ TG (selected, presumably HPRT⁻ clones). Cloning efficiency in the absence of TG was 15-48% and the frequency of mutant cells ranged from 2.5 × 10⁻⁶ to 2.0 × 10⁻⁵. TG-resistant clones were progressively expanded to >5 × 10⁷ cells by the intermittent addition of phytohaemagglutinin, irradiated allogeneic feeder lymphocytes from a female individual, and conditioned medium as a source of interleukin-2 (ref. 1). Genomic DNA was isolated from these monoclonal populations, digested with restriction endonucleases and electrophoresed on agarose gels; Southern analysis⁴ was performed using a full-length complementary DNA probe for human *hprt*⁵.

We have analysed 12 unselected clones and 21 selected clones from 7 individuals. In most cases, each genomic DNA sample was digested with three restriction enzymes (*Bam*HI, *Hind*III

Table 1 Major gene changes observed in 12 spontaneous HPRT⁻ mutants on Southern analysis

Clone	Donor	EcoRI				Change HindIII				BamHI			
		D	N	A	AN	D	N	A	AN	D	N	A	AN
2419 TGA	R.R.	+	+			+	+			+	+		
2419 TG5	R.R.	+				+				+			
2419 TG6	R.R.			+	+		+	+		←ND→			
2409 TG1	J.D.			+	+		+	+			+	+	
2409 TG2	J.D.			+	+		+	+			+	+	
2409 TG3	J.D.			+	+		+	+			+	+	
2412BTG1	J.W.	+	+			+				←ND→			
2412BTG2	J.W.	+		+	+	+	+			+	+		
AM416TG6	R.C.	+				+				+			
BC49 TGR	P.H.			+				+				+	
BC94 TGR	J.R.			+				+				+	
84810F	L.P.			+				+		←ND→			

Clones are listed in arbitrary order. The indication of a change (+) implies a change with respect to the unselected clonal pattern for that donor. Changes indicates are: D, deletion; N, novel band; A, amplified exon; AN, amplified novel band. ND, not done.

and *EcoRI*) for which the coding and pseudogene fragments have been identified⁶. In 12 of 21 (57%) mutant clones, heterogeneous changes in Southern patterns were identified when compared with unselected clones from the same individual. The unselected clones of each individual showed no changes and generally agreed with the gene fragments described by Patel *et al.*⁶. The changes observed in mutant clones include a variety of deletions, and the appearance of novel bands and presumed amplifications of intragenic coding fragments and novel bands (Fig. 1; Table 1). As expected, pseudogenes residing on the autosomes showed no changes in any clone. Southern patterns obtained from one donor showed the same additional gene bands present in all of three selected and three unselected clones examined. We interpret these additional bands present in all six clones to represent a previously undescribed polymorphism and, as this individual subsequently gave a history of gout, family studies are in progress. However, the three selected clones from this individual also showed amplifications of both novel and previously identified coding fragments (Fig. 2). Note that, although these three clones showed similar abnormalities, they were independently derived.

Although major gene alterations of the *hprt* gene were readily detected at the molecular level, parallel cytogenetic studies using block staining and G-banding at the 600-band level showed no structural abnormalities of the X-chromosome in either selected or unselected clones. The messenger RNA products of the mutated genes are being investigated.

It is possible that the results were caused by an *in vitro* rather than *in vivo* phenomenon. However, the experiments involved addition of TG immediately after removal and separation of the lymphocytes and it is known that immediate TG selection results in the elimination of *hprt* gene mutations produced *in vitro*, as there is not time for such mutations to be expressed as a phenotypic change⁷. Therefore, the selected clones must have arisen from lymphocytes which were already mutated *in vivo*. It is formally possible that the initial mutation for each clone did not involve a major gene alteration and that the observed major gene alterations resulted from the emergence *in vitro* of secondarily altered subclones which had a proliferative advantage and which overgrew the original cells of the clone. To test this possibility directly, a subcloning experiment was performed in which lymphocytes were cultured in 96 wells containing TG, each well being subcultured into 12 wells on day 4, at which time any HPRT⁻ lymphocytes would have undergone several divisions. After growth for 16 days in TG, two sets of subclones were chosen and expanded. Growth was present in 7 out of 12 wells in one set and 6 out of 12 wells in the other; the frequency of wells showing growth in each set indicated that the cells in most of the wells originated from a single cell and thus truly represented a subclone. The genomic DNA of each subclone was studied by Southern analysis and all subclones within each set were found to be identical; all of one set showed no change whereas all of the other (clone 84810F) showed the same *hprt* gene changes (amplification of exons). If major gene alterations develop during *in vitro* growth of lymphocytes, approximately half of each set of subclones should show abnormal Southern patterns and the abnormalities would be heterogeneous within a set. On the other hand, if major gene alterations are present *in vivo*, all subclones of a set would show either no abnormality or an abnormality which is identical for all members of a set. Our observation of the latter case provides direct evidence that the observed major gene alterations were present *in vivo* and had not arisen during clonal expansion *in vitro*.

The present experiments involved conditions of stringent TG selection and only mutations leading to a low or undetectable level of HPRT were likely to be recovered¹. For this class of mutations, our observations indicate that *in vivo* somatic mutations at the *hprt* locus are at least as likely to result from major gene changes involving several kilobases (kb) of DNA as from more site-specific events such as point mutations or minor deletions. Indeed, it is likely that the observed frequency of gene alterations (57%) underestimates the true frequency, as

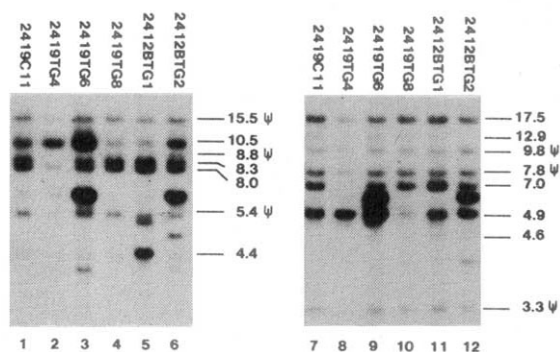


Fig. 1 Representative Southern transfer patterns of unselected (HPRT⁺) and selected (HPRT⁻) genomic DNA from two male donors (2419, 2412). Lanes 1–6, *EcoRI* digests; lanes 7–12, *HindIII* digests. Lane 1, unselected clone (2419 C11) showing normal enzyme digestion pattern with bands at 4.4 (not previously identified), 5.4 (Ψ), 8.0, 8.3, 8.8 (Ψ) faint in all tracks, 10.5 and 15.5 (Ψ) kb. Lane 2, selected clone (2419 TG4) showing deletion of 8.3-kb band containing exons towards the 5' end of the gene and a possible amplification of the 10.5-kb band. Lane 3, selected clone (2419 TG6) showing the appearance of a new amplified band of relative molecular mass 6.1 kb and amplification of the 10.5-kb band. Lane 4, selected clone (2419 TG8) which shows no evidence of Southern changes. Lane 5, selected clone (2412B TG1) showing amplification of a band at 4.4 kb and a new band at 5.2 kb. Lane 6, selected clone (2412B TG2) showing appearance of a new band at 6.1 kb, which may be amplified, and also a band at 4.8 kb. Lane 7, unselected clone (2419 C11) showing normal enzyme digestion pattern with bands at 3.3 (Ψ), 4.6 (not previously identified), 4.9, 7.0, 7.8 (Ψ), 9.8 (Ψ), 12.9 (not previously identified) and 17.5 kb. Lane 8, 2419 TG4 showing deletion of 7.0-kb band which hybridizes in unselected clones to the same cDNA fragment as that of the 8.3-kb band in the *EcoRI* digest (deleted in lane 2) and a possible amplification of the 4.9-kb band which correlates with the 10.5-kb band in the *EcoRI* digest of this clone. Lane 9, 2419 TG6 showing amplification of the 4.9-kb band and an amplified novel band at 6.4 kb. Lane 10, 2419 TG8 which shows no evidence of Southern changes. Lane 11, 2412B TG1 showing a possibly amplified band at 7.0 kb with this enzyme. Lane 12, 2412B TG2 showing appearance of a new band at 6.4 kb which may be amplified and a new band at 4.0 kb and a deletion of the 12.9-kb band.

Methods. For Southern transfers, 15 µg of genomic DNA extracted from cultured lymphocytes was digested with restriction enzymes, fractionated on 0.8% agarose gel and transferred to nitrocellulose by minor modifications of published procedures. Hybridization was carried out at 65 °C in 6×SSC 0.5% SDS, 5×Denhardt's, 0.01 M EDTA and 100 µg ml⁻¹ sonicated denatured herring sperm DNA following prehybridization in the mixture which was the same except that it contained 250 µg ml⁻¹ of the carrier DNA. The probe was a full-length human complementary DNA for *hprt* (insert cleaved from plasmid pHPT30 (ref. 6), *PstI/HhaI* cut, yielding a fragment of 873 base pairs (bp) which has, in addition to the entire coding region, 18 bp of 5'-untranslated and 201 bp of 3'-untranslated sequence). The probe was nick translated to a specific activity exceeding 2.5 × 10⁸ c.p.m. per µg and not less than 5 × 10⁶ c.p.m. ml⁻¹ hybridization mix was used. Hybridization was permitted for >16 h. Stringent washing was applied down to 0.5×SSC, 0.1% SDS, 1×Denhardt's at 65 °C. Autoradiographs were obtained on Ortho film using Lanex regular screens at -70 °C. Exposures were typically 48–72 h.

Southern analysis cannot resolve changes below a certain size unless a restriction site is involved. There is no reason to believe that events at the *hprt* locus are more or less frequent than at any other locus and we suggest that such changes in DNA are probably widespread throughout the genome.

It has been reported recently⁸ that major changes in Southern patterns were present in 5 of 28 patients with Lesch-Nyhan syndrome. The changes were heterogeneous and included deletions, new bands and, in one family, amplifications. This observation, together with our own, suggests that loss or amplification of genetic material may be an important mechanism for variability in both germline and somatic cells.

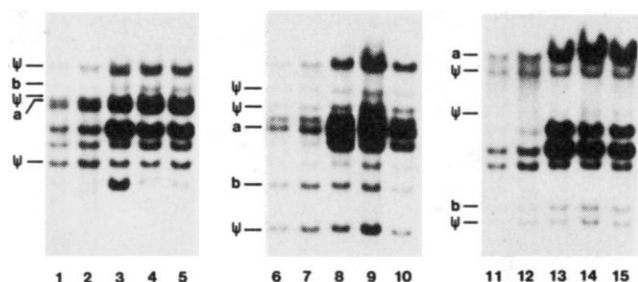


Fig. 2 Representative Southern transfer pattern of genomic DNA probed with cDNA for *hprt*. Two normal human male unselected (HPRT⁺) clones are shown, 2409 C1 (lanes 1, 6, 11) and 2409 C2 (lanes 2, 7, 12), and three selected (HPRT⁻) clones from the same individual, 2409 TG1 (lanes 3, 8, 13), 2409 TG2 (lanes 4, 9, 14) and 2409 TG3 (lanes 5, 10, 15). The enzymes used to digest DNA were *Eco*RI (lanes 1-5), *Hind*III (lanes 6-10) and *Bam*HI (lanes 11-15). The pseudogene allocations for *Eco*RI and *Hind*III digests are given in Fig. 1 legend. For *Bam*HI digests, the pseudogenes are shown at 3.4, 7.6, 14.5 and 25 kb (coincident with a coding region of the X-linked gene)⁶. Pseudogene fragments at 4.2 kb (*Eco*RI) and 2.0 kb (*Hind*III) do not show in this autoradiograph. It is clear that even allowing for somewhat greater loading of HPRT⁻ DNA onto the gel, there has been a significant amplification of certain parts of the *hprt* gene in each of three mutants. In particular, bands labelled 'a' are amplified (*Eco*RI, 8.3 kb; *Hind*III, 7.0 kb; *Bam*HI, 22 kb) whereas bands labelled 'b' are not (*Eco*RI, 10.5 kb; *Hind*III, 4.9 kb; *Bam*HI, 3.7 kb). The former are associated with exons 1 and 2 whereas the latter are associated with exons 3-6 (ref. 6). The simplest explanation of this is that the mutation has been occasioned by multiple reduplications of part of the gene containing exons 1 and 2. We also observe bands in these clones (HPRT⁺ and HPRT⁻) which are amplified in the mutants but which have not been previously assigned to the *hprt* gene. These results do not seem to be consistent with a restriction fragment length polymorphism and may therefore represent a non-pathological variant gene. We shall investigate the donor's family in more detail.

Direct evidence for the importance of somatic mutations in cancer comes from recent findings in familial and sporadic retinoblastoma⁹ and in Wilms' tumour¹⁰⁻¹³. In both disorders, one autosomal gene at a critical site is believed to be abnormal in all cells as the result of a familial or germ-cell mutation and the other normal allele is believed to have become lost through a chromosomal mutation associated with deletion, inactivation or recombination. Of the 22 cases of Wilms' tumour studied by molecular techniques, chromosomal mutations have been detected in 12, that is 55%, similar to the percentage of *hprt* mutations that we have observed showing major gene changes on Southern analysis. We believe that our observations represent a more general case in that there was no selection or drift during clonal expansion, such as presumably occurs during the development of a tumour phenotype. If, as our findings suggest, somatic mutations generate gene changes in a widespread fashion throughout the genome, loss or amplification of genetic material might be a phenomenon of more general importance in cancer, acting either by causing expression of a recessive abnormal allele as already discussed, by leading to a deletion of regions separating promoters or enhancers from proto-oncogenes and thus producing oncogene activation, or by causing amplification of sequences containing oncogenes, resulting in increases in oncogene products, as has been observed in some neuroblastoma lines¹⁴ and in the human promyelocytic HL60 cell line¹⁵.

Somatic mutations have also been postulated to be involved in the aetiology of ageing. Mutations at the *hprt* locus have been observed to accumulate with age¹⁶⁻¹⁸ and one can readily envisage that accumulating losses or amplifications of genetic material could lead to progressive deterioration of cellular function with age.

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Amplification and enhanced expression of the c-myc oncogene in mouse SEWA tumour cells

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SEWA tumour cells are derived from an osteosarcoma induced in an A.SW mouse by infection with polyoma virus¹. Cytogenetic analyses have revealed three different characteristic chromosomal abnormalities diagnostic for the presence of amplified genes: 'double minutes' (DMs), homogeneously staining chromosomal regions (HSRs) and C-bandless chromosomes (CMs; for review see ref. 2). DMs may undergo fluctuation in number depending on the conditions in which the cells grow. Their number usually increases after injection of cells into a mouse and often is reduced to undetectable levels when the cells are explanted back into tissue culture; when the cells are re-introduced into the mouse, they again acquire multiple DMs³. We show here that cells of SEWA lines carrying DMs, HSRs or CMs contain amplified copies of the proto-oncogene *c-myc* and enhanced levels of *c-myc* messenger RNA and *c-myc* protein. DMs or CMs are the sites of *c-myc* amplification in two different SEWA lines.

We used the SEWA cell lines 4031Tc3 (DMs), StamA (HSR) and Rec4Tc2 (CMs) to screen for amplification of oncogenes (for a genealogy of SEWA cells see ref. 2). Preliminary tests, in which we hybridized a ³²P-labelled complementary DNA synthesized from poly(A)⁺ RNA to nitrocellulose filters containing different oncogene DNAs⁴, had provided evidence for abundant *c-myc* mRNA in SEWA cells (data not shown). We therefore investigated whether *c-myc* is amplified in SEWA cells. Total genomic DNA was cleaved with restriction endonucleases, fractionated on agarose gels and blotted to nitrocellulose filters to which ³²P-labelled *c-myc* probe was hybridized. For comparison, we tested DNA from testes of the A.SW mouse and DNA from human cells of the COLO 320-DM line, in which an apparently normal *c-myc* allele and a mutant allele rearranged at the 5' end are each amplified ~25-fold (ref. 5 and M.S. *et al.*, manuscript in preparation). The autoradiographic signal

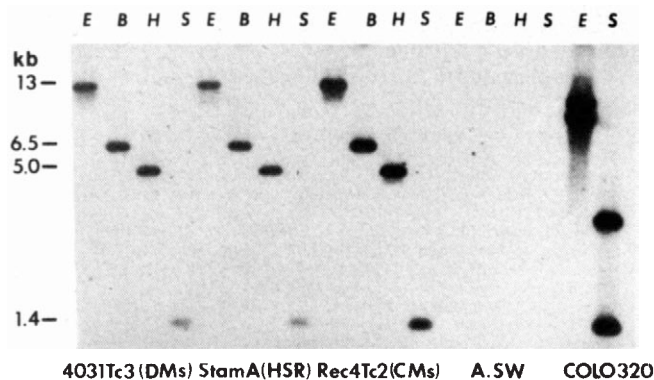


Fig. 1 Analysis of *c-myc* in the DNA of SEWA cell lines. NIH 3T3 and COLO 320-HSR and -DM cells served as controls. Approximately 10 μ g of DNA was digested with restriction endonucleases (E, *EcoRI*; B, *BamHI*; H, *HindIII*; S, *SacI*), fractionated on agarose gel and transferred to nitrocellulose filter, to which 32 P-labelled *PstI* fragment of exon II of human *c-myc* was hybridized. COLO 320 DNA digested with *EcoRI* was from the DM subline, DNA digested with *SacI* from the HSR subline. DNAs were analysed with a single-copy gene probe (*Ha-ras*) to show that approximately equal amounts of DNA of each line were being used (result not shown).

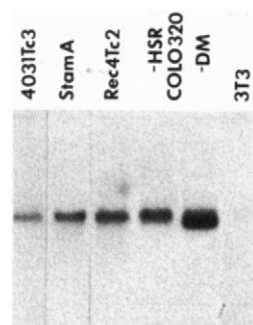


Fig. 2 Characterization of RNA transcribed from *c-myc* in SEWA cells. NIH 3T3 cells and COLO 320-HSR and -DM cells were used for comparison. Poly(A)⁺ RNA (5 μ g per lane) was fractionated on agarose gel in the presence of 2.2 M formaldehyde, transferred to nitrocellulose filters and hybridized to *c-myc* probe.

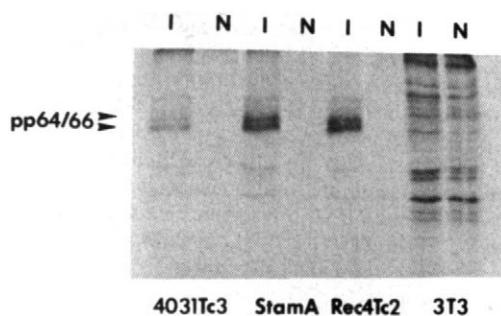


Fig. 3 Immunoprecipitation of pp64/pp66-*c-myc* from SEWA cells. NIH 3T3 cells were used for comparison. I, immune serum from rabbits immunized with a peptide from the C-terminus of human *c-myc*⁶; N, non-immune serum collected before immunization.

obtained for the DNA of all three SEWA lines tested was much stronger than that obtained for the DNA from the A.SW mouse. SEWA cell lines 4031Tc3 and StamA contained ~15–20-fold and line Rec4Tc2 ~20–30-fold amplification of *c-myc* (Fig. 1). The size of the DNA fragments detected with the *c-myc* probe was the same in all three SEWA lines and in the normal mouse DNA fragment, indicating that the amplified *c-myc* gene in the SEWA calls has not undergone major rearrangement.

We next used RNA blotting procedures to test whether *c-myc* amplification is associated with a proportional enhancement of

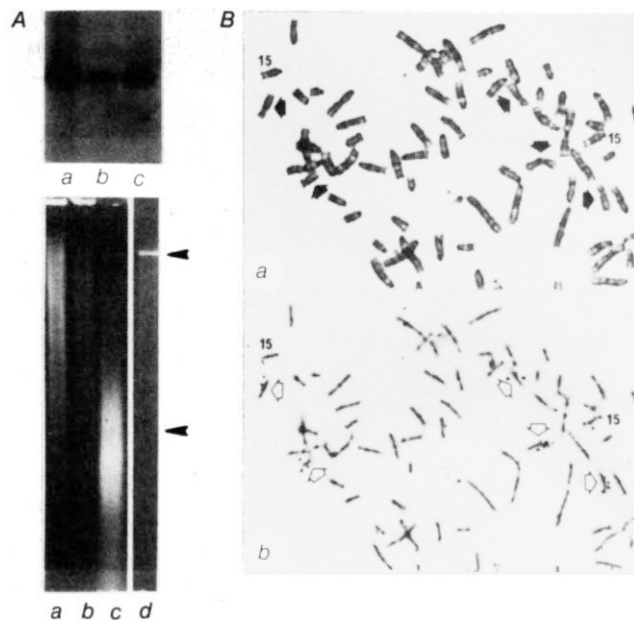


Fig. 4 Presence of amplified *c-myc* in DMs and CMs. **A**, Localization in DMs. Approximately 10 μ g of DNA from intact nuclei and undisturbed cells (lane a), 5 μ g of the fraction containing the macrochromosomes (lane b) and an estimated 0.5 μ g of the DM fraction (lane d) were fractionated on an agarose gel and blotted onto nitrocellulose filter. The upper part of the figure shows the result obtained by hybridizing *myc* probe to the filter, the lower part shows the same gel stained with ethidium bromide. The ethidium bromide stained gel displays the enrichment for specific DNA sequences, presumably repetitive DNA (lane d, arrowheads indicate bands due to DNA fragments ~16 and 0.8 kb in size), which do not hybridize with *c-myc* probe, however. The intense fluorescence in lane c is probably due to the presence of RNA contained in ribosomes which co-sediment with DMs; the signal disappears following treatment of the gel with pancreatic ribonuclease (lane e). **B**, Localization in CMs. SEWAC-3 (a derivative of Rec4) containing CMs was used. Arrows indicate CMs. **a**, Metaphase spread after trypsin/Giemsa treatment. **b**, The same spread after destaining in methanol and *in situ* hybridization to 32 P-labelled *c-myc* probe. Note that four of the silver grains in the figure which are not situated over CMs are localized to chromosome 15 (indicated), which contains the *c-myc* gene.

Methods. Approximately 10^8 exponentially growing cells were arrested in metaphase by colchicine (0.05 μ g ml⁻¹ for 6 h) and collected. Chromosomes were released by gently drawing the cell suspension up and down three times through a 25-gauge needle in chromosome isolation buffer (15 mM Tris-HCl, 0.2 mM spermine, 0.5 mM spermidine, 2 mM EDTA, 0.5 mM EGTA, 80 mM KCl, 20 mM NaCl, 14 mM mercaptoethanol, pH 7.2)⁹ containing 0.34 M sucrose, 0.1% digitonin and 0.5 M 2-methyl-2,4-pentanediol (Kodak). Nuclei and undisturbed cells were pelleted for 10 min at 500 r.p.m. in a JS 4.2 rotor (Beckmann), and subsequently the macrochromosomes were removed by repeated centrifugation at 4,000 r.p.m. The final supernatant, which on the basis of inspection by light microscopy was virtually free of macrochromosomes, was centrifuged at 25,000 r.p.m. (SW 50.1, Beckmann) for 45 min to pellet the DMs. DNA was prepared from the intact nuclei and undisturbed cells, from the pellets containing the macrochromosomes and from the pellets containing the DMs, and was digested with *EcoRI*.

the level of *c-myc* mRNA. For comparison, we tested polyadenylated RNA from NIH 3T3 cells and from cells of the COLO 320-HSR and -DM lines containing ~25-fold enhanced levels of mRNA⁵. COLO 320-HSR cells have an mRNA of normal size, ~2.5 kilobases (kb), and COLO 320-DM cells carry predominantly a 2.3-kb *c-myc* mRNA as the result of *c-myc* rearrangement. The size of the *c-myc* mRNA in the SEWA cells and the COLO 320-HSR cells was indistinguishable (Fig. 2). Levels of *c-myc* mRNA in lines 4031Tc3, StamA and Rec4Tc2 were elevated 5–10-fold, 15-fold and 25-fold, respectively, com-

pared with NIH 3T3 cells; the level in Rec4Tc2 cells approached that found in the COLO 320 cells. The mouse *c-myc* gene encodes two forms of the *c-myc* protein, with relative molecular masses of 64,000 and 66,000 (pp64/66)⁶. Immunoprecipitation with anti-*myc* serum⁶ showed that the level of *c-myc* protein was greatly increased and that the protein itself was unaltered (Fig. 3).

We have shown recently that DMs and HSRs in several human and murine tumour cells contain amplified cellular oncogenes (for a review see ref. 7). To test whether amplified *c-myc* is localized in DMs of cells of SEWA line 4031Tc3, a chromosomal fraction enriched for DMs was prepared according to the procedure previously used to localize *c-Ki-ras* in DMs⁴. DNA was isolated from intact nuclei and undisrupted cells, from pellets containing the macrochromosomes and from pellets containing the DMs. It was digested with restriction endonuclease *EcoRI*, fractionated on agarose gels and transferred to nitrocellulose paper to which ³²P-labelled *c-myc* probe was then hybridized. The autoradiographic signal obtained using ~0.5 µg of DNA from the DM fraction (Fig. 4A, upper part, lane c) was much more intense than that obtained for 5.0 µg DNA from the macrochromosomes (lane b), and only slightly less intense than that seen for 10 µg DNA isolated from total nuclei and undisrupted cells (lane a). These results indicate that the DM fraction is enriched for *c-myc* and that DMs are the site of *c-myc* amplification. In another experiment, when SEWAC-3 cells (a derivative of SEWAC4 containing CMs) were hybridized *in situ* to ³H-labelled *c-myc* probe, the average number of grains found over CMs was 10 times greater than that over other chromosomes (Fig. 4B, lane b), indicating that CMs are the site of amplified *c-myc*.

The generation of DMs following transfer of SEWA cells from *in vitro* culture into the mouse, and the loss of DMs following re-transfer of the cells into tissue culture has long been thought to reflect changes in the copy number of genes involved in malignancy⁸. The present results show that *c-myc* is amplified and overexpressed in SEWA cells and, at least in cells of lines 4031Tc3 and C-3, is probably localized in DMs and CMs, respectively. Although the amplified unit of DNA probably contains considerable genetic information in addition to the *c-myc* gene, it is possible that enhancement of expression of *c-myc* after amplification confers on the host cells a selective advantage for tumour formation in the natural host. This idea is supported by our finding that the degree of amplification of *c-myc* observed in different SEWA lines is correlated with the rate at which tumours develop when the cells are injected into a mouse (T.M. *et al.*, manuscript in preparation). Southern blotting procedures revealed that SEWA cells, which are derived from a polyoma virus-induced tumour¹, contain three to five copies of polyoma virus (data not shown). It is unclear which function, if any, these polyoma sequences contribute to the malignant phenotype in the presence of amplified *c-myc* gene. We are now analysing the possible significance of *c-myc* amplification in SEWA cells in more detail.

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Size variation in chromosomes from independent cultured isolates of *Plasmodium falciparum*

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The complexity of the life cycle of the protozoan malaria parasite *Plasmodium falciparum* has hindered genetic analysis¹; even the number of chromosomes in *P. falciparum* is uncertain². The blood stages of rodent malaria parasites are haploid^{1,3} and hybridization with cloned complementary DNAs similarly suggests a haploid genome in *P. falciparum* blood stages (ref. 4 and our unpublished results). A novel approach to karyoptic and linkage analysis in *P. falciparum* has been provided recently by the technique of pulsed-field gradient (PFG) gel electrophoresis⁵, which allows the fractionation of DNA molecules of 30–3,000 kilobases (kb), a range including the sizes of intact chromosomal DNA molecules from eukaryotes such as yeast⁵ and trypanosomatids⁶. We describe here the fractionation by PFG electrophoresis of chromosomal DNA molecules from *P. falciparum* into at least seven discrete species which vary in size by up to 20% between different isolates. Several genes for *P. falciparum* antigens which contain repetitive sequences are located on different chromosomes. Surprisingly, two of the chromosomes seem to contain the same sequences.

Intact chromosomal DNA^{5,6} was prepared by immobilizing the organisms in low-gelling point agarose and then deproteinizing *in situ* (see Fig. 1 legend). The chromosomal DNA molecules were subsequently separated by PFG electrophoresis in a 1.5% agarose gel. In this procedure, an electric field is applied in alternate pulses first in one direction, then in a direction oriented 90° to the first. Because the DNA molecules are considerably longer than the pore size of the gel, they must align with the field in order to migrate 'end-on' through the gel. As the time to re-orient is a function of size, the molecules can be resolved if the pulse time is tuned to an appropriate frequency⁵. We found that the optimal pulse time for *P. falciparum* chromosomes was ~80 s. The separated chromosomal DNA molecules can then be stained with ethidium bromide (EtBr) and blotted to nitrocellulose for gene localization by hybridization⁵.

Fractionation of the chromosomes of cloned *P. falciparum* line E12 (ref. 7), derived from the Papua New Guinea isolate FCQ27/PNG (FC27), is shown in Fig. 1a; E12 and FC27 were identical (data not shown). Migration is nonlinear because of the focusing effect of the asymmetrical electrode array⁵. Hence, the DNA molecules in each track lie on a curve, and equivalent bands in successive tracks form a curve across the gel. In the region of highest mobility the DNA formed a smear (S in Fig. 1). Hybridization data (see below) demonstrated that the smear contains fragments of the larger chromosomes, but this does not exclude the presence of other components, for example, mitochondrial DNA or 'minichromosomes'.

In the central region, four sharp bands, designated chromosomes 1–4, were clearly resolved (Fig. 1a). To estimate their sizes, yeast chromosomes were used as markers (Fig. 1c). The 17 yeast chromosomes were resolved into about 11 bands (Fig. 1c) plus a lower band of mitochondrial (YM) DNA. Chromosomes 1–4 of *P. falciparum* clone E12 migrated in the same range as the larger yeast chromosomes. Taking the sizes of the three smallest yeast chromosomes to be about 0.3, 0.5 and 0.7 megabases (Mb) and the largest to be about 2.0 Mb (ref. 5), we estimated that E12 chromosomes 1–4 are roughly 0.8, 1.2, 1.6 and 1.8 Mb in size, respectively. From their discrete sizes and from hybridization data (see below) showing that they contain different sequences, we conclude that they are intact chromosomes.

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Nearer the top of the gel, bands were less clearly resolved (see Figs 1–3). Hybridization data (see below) indicate that there are at least three chromosomes in this region. Chromosomes 5 and 6 were usually evident as discrete bands while chromosome 7 smeared from the well, partially overlapping chromosome 6. By extrapolation, we have assigned chromo-

somes 5–7 nominal sizes of 2.2, 2.6 and 3.0 Mb respectively (Fig. 4), but there are no accurately known size markers in this range. Some EtBr-staining material consistently remained in the well. Hybridization suggested that it contains a poorly reproducible proportion of all the chromosomes, presumably trapped in debris. The possibility of an additional chromosome(s) in this region cannot be excluded.

Comparison of chromosomes from *P. falciparum* clone E12 with those of isolates NF7 from Ghana and K1 from Thailand revealed striking size polymorphisms (Fig. 1b, d). Chromosome 1, the smallest chromosome of each isolate, differed in size between the three isolates, as can be seen by comparing the alternate tracks of E12 and NF7 (Fig. 1b) and of E12 and K1 (Fig. 1d). The sizes of chromosome 1 were estimated to be ~0.8 Mb (E12), 0.9 Mb (NF7) and 1.0 Mb (K1).

No chromosome of a size similar to E12 chromosome 2 was present in NF7 or K1, whereas both NF7 and K1 possess a chromosome intermediate in size between E12 chromosomes 4 and 5. All three isolates have chromosomes similar in size to E12 chromosomes 3 and 4, but hybridization indicated that this apparent similarity is misleading (see below). All three isolates have chromosomes apparently equivalent in size to E12 chromosomes 5–7, but the resolution is poor in this region.

To clarify the relationships between individual chromosomes in the three isolates, we preparatively isolated FC27 chromosomes by PFG electrophoresis, digested each with *EcoRI*, subcloned the fragments into pUC9 (ref. 8) and hybridized individual chromosome-specific plasmids to blots of E12, NF7 and K1 chromosomes separated by PFG electrophoresis (Fig. 2). Two independent probes that hybridized only to chromosome 1 in E12 also hybridized only to the smallest chromosome in NF7 and K1 (data not shown), as did a cloned cDNA (see below). Hence, the smallest chromosomes of each isolate are counterparts, designated chromosome 1. Hybridization with a probe (pF2.1) specific for E12 chromosome 2 (Fig. 2b) revealed that the corresponding chromosome in K1 was much larger (Fig. 2b). The same results were obtained with this probe when E12 and NF7 were compared (data not shown). Hence, the second smallest chromosome in each isolate is a counterpart of E12

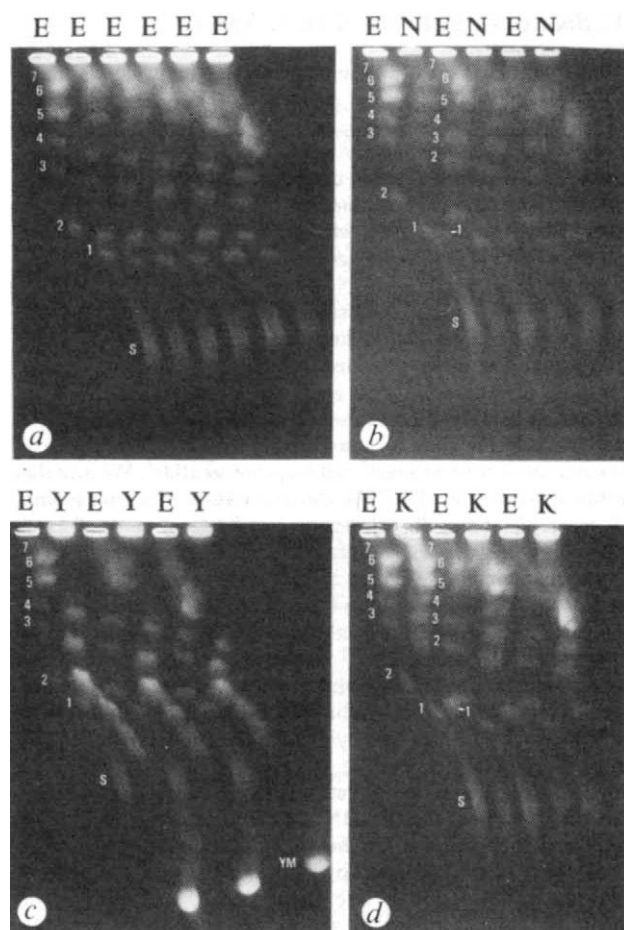


Fig. 1 Fractionation of *P. falciparum* chromosomes by pulsed field gradient gel electrophoresis. In a–d the chromosomes were from clone E12 (E) derived from *P. falciparum* isolate FC27, isolate NF7 (N) or K1 (K). The marker chromosomes (Y in panel c) were from yeast (*Saccharomyces cerevisiae*, strain J69b1). The samples were run in for 2 h at 200 V in the north–south direction, then electrophoresed for 22 h with 80-s pulses, 250 V in the north–south direction (top–bottom) and 100 V in the east–west direction (left–right) at 18 °C, and stained with EtBr.

Methods. *P. falciparum* isolates were maintained *in vitro*¹⁶ on human erythrocytes. Cultures were collected at 5–10% parasitaemia and the parasites were concentrated by lysing the red cells with saponin¹⁷, followed by two washes. The parasites were resuspended in phosphate-buffered saline at $\sim 6 \times 10^9$ organisms per ml, and added to an equal volume of molten 2% low-gelling agarose in saline at 37 °C, and cast into $1.6 \times 5 \times 5$ mm blocks⁵. The blocks were treated with 0.5 M EDTA/0.01 M Tris/1% Sarkosyl/2 mg ml⁻¹ proteinase K for 48 h at 42 °C⁵ and stored at 4 °C. Before electrophoresis the blocks were cut into strips $\sim 1.6 \times 1.0 \times 5$ mm, soaked in $0.9 \times$ TBE⁵ for 1 h and then inserted into the slots of a 1.5% agarose (Marine Colloids, type ME) gel in $0.9 \times$ TBE and sealed into the slots with 1% low-gelling agarose. Yeast chromosomes were prepared as described previously⁵. The PFG apparatus was constructed essentially as described elsewhere⁵, with 15 diode-isolated electrodes in the west, north and east sides and a single electrode in the south-west corner. The gel ($225 \times 215 \times 5$ mm) was supported on 2-mm-thick Perspex blocks, to allow cooling by flow of the electrolyte under as well as over the gel. Cooling was achieved by a tap-water heat exchanger and the electrolyte ($0.9 \times$ TBE) was recirculated between buffer reservoirs at the north and south ends at 600 ml min⁻¹ with a solution metering pump.

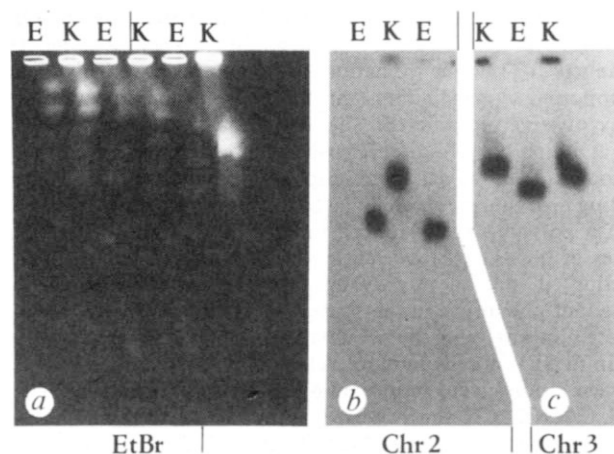
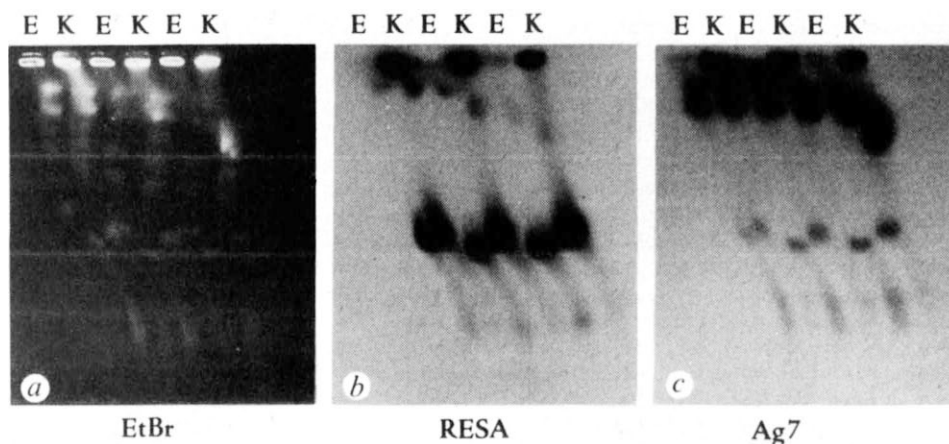


Fig. 2 Relationships between the chromosomes of *P. falciparum* cloned line E12 (E) and isolate K1 (K). Chromosomes were fractionated as in Fig. 1 and stained with EtBr (a). The DNA was then blotted to a nitrocellulose filter which was cut into two parts. The left and right parts were hybridized with a cloned FC27 chromosome 2 (b) and chromosome 3 (c) probe.

Methods. FC27 chromosomes were fractionated as in Fig. 1a. Bands corresponding to each chromosome were sliced from the preparative gel, electrophoresed into and then extracted from low-gelling agarose. Chromosome-specific hybridization probes were generated using *EcoRI* digestion of DNA from these individual chromosome bands and ligation of fragments into the plasmid vector pUC9 (ref. 8). Fragments released from the resulting clones pF2.1 and pF3.3 by *EcoRI* digestion were gel-purified, nick-translated and used directly as hybridization probes as described previously¹⁸.

Fig. 3 Chromosomal locations of genes for repetitive antigens. Chromosomes from E12 and K1 were fractionated as in Fig. 1 and stained with EtBr (a). The DNA was then blotted to nitrocellulose filters and hybridized with a RESA probe (clone Ag13, from ref. 12) (b). After autoradiography, the filter from b was rehybridized with a fivefold greater concentration of an Ag7 (ref. 18) probe and autoradiographed again (c). Hybridization of both these probes to the origin for K1 is probably an artefact resulting from trapped DNA (see also Fig. 2c).



chromosome 2, even though it differs in size by about 0.3 Mb (that is, ~20%) between isolates. Similarly, three independent probes (pF3.3, pF3.9 and pF3.1S) specific for E12 chromosome 3 (Fig. 2c and data not shown) revealed that the corresponding chromosome in K1 was considerably larger. Thus, the third smallest chromosome in each isolate is a counterpart of E12 chromosome 3, even though E12 chromosome 3 is considerably smaller (by ~0.2 Mb) than that of K1 or NF7.

K1 and NF7 each contain a chromosome that is intermediate in size between E12 chromosomes 4 and 5. We presume that this chromosome 4 corresponds to chromosome 4 of E12, which is about 0.2 Mb smaller, because we have accounted above for chromosomes 1-3 in each isolate, and hybridization with other DNA probes (see below) shows that chromosomes 5-7 are equivalent (Fig. 4). Remarkably, all four chromosomes that can be clearly resolved are considerably larger in NF7 and K1 than are their counterparts in E12 and FC27.

Several antigens of *P. falciparum* are unusual proteins containing multiple repeats of oligopeptide sequences^{4,9-14}. Assignment of these genes by hybridization to separated chromosomes would provide an approach to analysis of the genetic organization of genes coding for these repetitive antigens. Hybridization of a cloned sequence encoding RESA, a repetitive *P. falciparum* antigen located at the membrane of the newly infected erythrocyte¹², indicates that the RESA gene is located on the polymorphic chromosome 1 in both E12 and K1 (Fig. 3b). The RESA gene probe also hybridized to chromosome 7 but to a much lower extent, in agreement with our observation that several restriction fragments cross-hybridized to RESA¹². The gene for a second repetitive antigen, an isolate-specific secreted protein designated the S-antigen⁹, was also located on chromosome 7 (data not shown), providing further compelling evidence that chromosome 7 is indeed distinct from chromosomes 5 and 6.

Figure 3c shows hybridization of a cDNA probe encoding a third repetitive antigen designated Ag7, which apparently becomes associated with the schizont-infected erythrocyte and contains hexapeptide repeats (our unpublished observations). The Ag7 probe hybridized to both chromosomes 5 and 6 in E12 and K1 (Fig. 3c) and in NF7 (data not shown). Its localization on two chromosomes was surprising, as the same Ag7 probe hybridized to a single polymorphic restriction fragment after digestion with *Eco*R1, *Aha*III or *Hind*III.

A cDNA probe encoding a fourth repetitive antigen, designated FIRA (for *Falciparum* interspersed repeat antigen, ref. 4) also hybridized to both chromosomes 5 and 6 in E12, K1 and NF7 (data not shown). Again, conventional Southern hybridization had suggested a single gene in each isolate⁴, with polymorphisms between isolates. Three other cloned sequences derived from isolated chromosomes (pF5.9, pF5.14 and pF5.17, data not shown) all hybridized to chromosome 5 as well as chromosome 6. Hence, all five sequences that hybridized to chromosome 5 also hybridized to chromosome 6, in each of the isolates including clone E12. This is a paradoxical observation because Ag7 and FIRA are both highly polymorphic (ref. 4 and

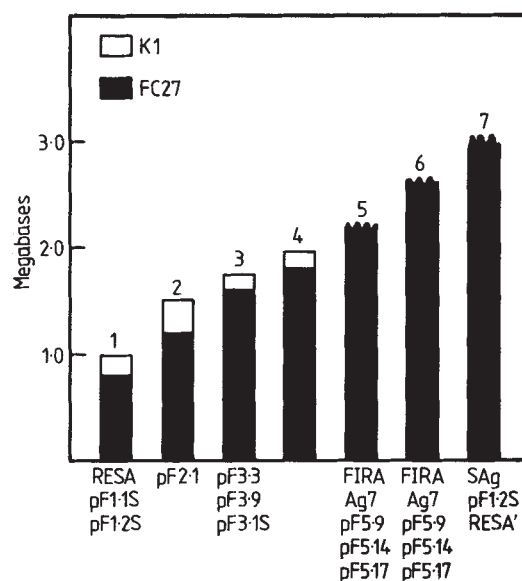


Fig. 4 Karyotypes of FC27 and K1. The heights of the bars represent the approximate sizes of chromosomes 1-7; the wavy lines depicting chromosomes 5-7 imply considerably less accuracy in these sizes. The scale (in Mb) is indicated at the left. Hybridization results are summarized below each chromosome. NF7 was indistinguishable from K1, except that chromosome 1 was ~0.9 Mb. Probes designated pF were generated from isolated chromosomes. RESA' indicates cross-hybridization (Fig. 3b).

our unpublished observations). In seven different isolates, including those used here, single restriction fragments were detected by each probe, although the size of these fragments differed between isolates. Hence, each isolate was apparently homozygous at each of these two highly polymorphic loci, so we concluded that the blood stages of *P. falciparum* are haploid, in agreement with genetic studies¹. However, the single alleles of Ag7 and FIRA present in each of these isolates seem to each reside on both chromosomes 5 and 6. On the basis of EtBr binding, chromosomes 5 and 6 appear to be equimolar and present at the same copy number (presumably 1 per cell) as chromosomes 1-4. A possible resolution of this paradox could be that chromosome 5 is an exact but incomplete copy of chromosome 6. Other variations of this model are obviously possible, the crucial point being that *P. falciparum* appears to be haploid for chromosomes 1-4 and diploid for a chromosome detected as 5-6 because the two copies are of different sizes. At present, we have no independent method to confirm this unexpected observation. It may be an artefact of this new technique, perhaps resulting from chromosome breakage at a specific site.

The data described here illustrate the value of PFG electrophoresis in cytogenetic analysis of a protozoan parasite that

otherwise is genetically intractable. These results show clearly that genes for repetitive antigens are distributed among the chromosomes of *P. falciparum* rather than clustered on one chromosome. The karyotype of FC27 was remarkably different from those of NF7 and K1, in that chromosomes 1–4 were all considerably smaller, and karyotyping by PFG electrophoresis has enabled us to distinguish these three and another two isolates (7G8 and V1) examined so far.

The phenomenon of uniformly larger chromosomes in one isolate compared with another is reminiscent of differences in the chromosomes of closely related grasshoppers which differ in their content of repetitive heterochromatic sequences¹⁵. However, deletions occurring *in vitro* could confer a growth advantage and so cultured lines may be rapidly overtaken by such variants. The genetic differences underlying these karyotype differences may be of considerable importance, both clinically and in the efficacy of any future vaccines.

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Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate

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Post-meiotic segregation of alleles, which is seen, for example, in the 5:3 distribution of alleles in the products of a single meiosis in fungi, has been thought to be due to the non-repair of heteroduplex regions formed during genetic recombination. In current models of genetic recombination, heteroduplex DNA is formed either as the primary intermediate generated by two interacting non-sister chromatids¹ or as a short region flanking a double-stranded gap². The frequency of post-meiotic segregation differs for different alleles, and this is presumed to reflect the varying efficiencies with which different types of mismatches in the heteroduplex are repaired. To gain some insight into this process, we have now determined the nucleotide sequences of various yeast alleles with different post-meiotic segregation frequencies and compared the mismatches predicted to occur in heteroduplexes of these alleles with wild-type DNA with those repaired with varying efficiency in bacterial systems. A striking correlation is observed, with the mismatches predicted for high post-meiotic segregation frequency alleles being similar to mismatches repaired with low efficiency in bacteria. These results support the view that post-meiotic segregation frequency reflects heteroduplex repair efficiency and the contention that meiotic gene conversion is the result of the successful repair of heteroduplex mismatches.

The cloning of the *ADE8* gene has been reported previously³. Two methods were used to clone the *ade8-18* allele⁴, both in an *ade8-18* host; these were mitotic gap repair⁵ and meiotic gene conversion of the *ADE8* plasmid. Figure 1a shows a detailed restriction map defining the fragments subcloned for dideoxy sequencing⁶ while Fig. 1b displays the nucleotide sequence of the *ADE8* gene. The *ade8-18* mutation is a 38-base-pair (bp) deletion removing the third position of codon 88 to the first position of codon 101, inclusively. This accounts for the observed non-revertibility of the allele⁷. The *ADE8* sequence codes for a 214-amino-acid protein, in close agreement with the predicted 232-amino-acid length based on meiotic and X-ray mapping data⁷. The deletion removes a unique *Xho*I restriction

Table 1 Shared sequences in *ADE8* and *ARG4**

Sequence	Location in <i>ADE8</i>	Location in <i>ARG4</i>
AAGATCC	619	221
AAGGCAA	614	29
AAGTTT	551, 1,001, 1,134	231
ACAAAGG	548, 575	526
AGATCCC	620	371
ATGTCAG	817	38
CAAAGGG	576, 953	527
CGATATT	741	343
GATCCTT	700	89
GCGGGCT	686	176
AAGTTTGT	551	231
TTTGAGAAA	629	400
TACAAAGGG	574	525

Numbering for *ADE8* begins with the first nucleotide in Fig. 1b. For reference, nucleotides 646–683 are deleted in the *ade8-18* mutation. Numbering for *ARG4* begins with the first nucleotide in Fig. 2. For reference, *arg4-17* is at nucleotide 164 and *arg4-16* is at nucleotide 378.

site in the coding sequence; this site is absent in both independently isolated *ade8-18* plasmids.

Figure 2 shows the nucleotide sequence of the 566-bp *ARG4* *Sst*I fragment. The ochre mutation *arg4-17*, with low post-meiotic segregation, is an A to T transversion in the first position of codon 43. The plasmids pSZ524 and pSZ524-17 (provided by T. Orr-Weaver and J. Szostak) were the sources of *ARG4* and *arg4-17* DNA. The *arg4-16* mutation, showing high post-meiotic segregation, is a G to C transversion in the second position of codon 114, substituting proline for arginine. The *arg4-16* allele was cloned by mitotic gap repair⁵. The *ARG4* sequence in Fig. 2 agrees with that recently published⁸ except at position 346, where we read T instead of G, resulting in aspartate rather than glutamate.

The finding that heterozygosity for the *ade8-18* 38-bp deletion displays high post-meiotic segregation (54% among aberrant tetrads) is unexpected. Previous studies of *HIS4* deletions showed that a 400-bp deletion (*his4-Δ15*) displays gene conversion but no post-meiotic segregation, although a presumed point mutation (*his4-4°*) in the deleted region displays similar non-mendelian segregation levels, but 10.3% post-meiotic segregation⁹. Single-base insertions also seem not to result in high post-meiotic segregation (Table 2). The relationship between deletion size and the frequency of post-meiotic segregation warrants further investigation.

The high frequency of post-meiotic segregation which is characteristic of the *ade8-18* allele does not seem to be a simple

Table 2 Mismatch specificity of post-meiotic segregation in yeast and transformation in *Streptococcus*

<i>S. cerevisiae</i>			<i>S. pneumoniae</i>		
Diploid	Mismatch	Post-meiotic segregation frequency	Efficiency class	Mismatch	Relative efficiency
<i>ADE8/ade8-18</i>	38-bp deletion	54.1%	VHE	33-bp deletion	0.86–1.06
<i>ARG4/arg4-16</i>	C/C or G/G	32.5%	HE	C/C or G/G	0.49–0.64
				A/G or C/T	
<i>ARG4/arg4-17^o</i>	A/A or T/T	5.3%	IE	A/A or T/T	0.17–0.26
<i>arg4-17^o/arg4-17^o</i>	A/C or G/T	4.2%	LE	A/C or G/T	0.0026–0.085
<i>HIS4/his4-519</i>	G insertion	0			
<i>HIS4/his4-518</i>	C insertion	0			

The frequencies of post-meiotic segregation are from ref. 9, except *his4-519* (197 tetrads)¹⁸ and *his4-518* (712 tetrads, S.F. and K.L., unpublished results). The *his4-518* sequence is from M. Culbertson (personal communication): ACC→ACCC and the *his4-519* sequence is from ref. 19. The nomenclature for *S. pneumoniae* efficiency classes is from ref. 16, where one to four sequenced examples of each class were reported. The relative transformation efficiencies are from Lacks¹⁵. Large deletions¹⁵ and presumed frameshifts²⁰ are usually LE markers in *Streptococcus*. VHE, very high efficiency; HE, high efficiency; IE, intermediate efficiency; LE, low efficiency.

interest (*ADE8 HpaI/SalI* fragment and *ARG4 SstI* fragment) (see Table 1). Although one of the sequences abuts on the *arg4-16* site and is also represented only 19 bases away from the *ade8-18* breakpoint, another sequence is equally close to *ade8-18* and the low post-meiotic segregation *arg4-17* site. No new homology is generated by any of the mutations studied in these regions. Therefore, our data neither support nor rule out the presence of a pause site near the two high post-meiotic segregation alleles.

Table 2 lists the *Saccharomyces cerevisiae* hDNA mismatches generated by the alleles whose sequences were determined in the present study. No other data on mismatch repair efficiencies for specific mismatches in *S. cerevisiae* exist. However, post-meiotic segregation is observed for specific G to C transversions creating C/C or G/G mismatches during meiotic conversion of transfer RNA genes in *Schizosaccharomyces pombe*¹¹. Analysis of two bacteriophage T4 frameshift mutations which could have arisen through hDNA repair on a quasipalindromic sequence containing a two-base bulge (that is, an insertion relative to the other 'strand') and three sets of base-pair mismatches, G/G, T/G and C/C, yielded one mutant in which all three base pair mismatches had been corrected along with the bulge, while in the other mutant only the C/C mismatch survived the correction process¹².

There is a striking correlation between the *S. cerevisiae* data shown in Table 2 and the relative transformation efficiencies of various markers in *Streptococcus pneumoniae*. Exogenous DNA is converted to single-stranded DNA upon or concomitant with entry into the *S. pneumoniae* cell¹³. The DNA single strands are then taken up by host DNA¹³, presumably resulting in hDNA and D-loops in homologous regions. Marker transformation efficiency is interpreted as being a function of the probability of hDNA mismatch recognition¹⁴. Once recognition occurs, incoming information is excised. Thus, transformation efficiency is inversely related to hDNA mismatch recognition¹⁴. The right half of Table 2 presents the four identified transformation efficiency classes¹⁵ and the potential hDNA mismatches arising from sequenced examples¹⁶. Comparisons within Table 2 reveal that the frequency of post-meiotic segregation in yeast and transformation efficiency in *Streptococcus* seem to be based on similarly specific hDNA mismatch correction. Correction of individual base pair mismatches by the methylation-directed system in *Escherichia coli* is also similar. For example, C/C, A/G and C/T are inefficiently corrected, A/A is fairly efficiently corrected and A/C and G/T are well corrected¹⁷.

Based on the above correlations, we are constructing diploid strains harbouring all six possible pairs of mismatches at a single site. These will provide critical information concerning the nature of the meiotic recombination intermediate. If the intermediate is primarily hDNA, the frequency of post-meiotic segregation should vary with the mismatch generated. However, if

the intermediate is primarily a double-stranded gap, post-meiotic segregation frequency will depend only on the sequence present on the gapped or recipient chromatid. In this instance, similar post-meiotic segregation frequencies would be expected for all six diploids unless a particular mutant allele creates a pause site not present in the wild-type strain.

In conclusion, we have presented data which comprise a framework for a molecular analysis of the meiotic recombination intermediate. If the intermediate is a double-stranded gap, 54% of the gaps formed near the *ade8-18* site must have a hDNA segment of at least 38 bp, perhaps due to a pause site. On the other hand, we find that the frequency of post-meiotic segregation is correlated with the nature of specific hDNA mismatches analysed and our data therefore suggest a hDNA intermediate. In fact, data for two point crosses at *ARG4* suggest that the A/A or T/T mismatch generated by *arg4-17^o* can promote correction of the *arg4-16* C/C or G/G mismatch⁹. Thus, sites separated by 213 bp can be involved in the same hDNA region. If further experiments confirm that segregation frequency is controlled by the structure of the mismatch itself, rather than the sequence environment, it will be possible to estimate more accurately the minimum contribution of hDNA to the meiotic recombination intermediate.

We thank T. Orr-Weaver and J. Szostak for supplying plasmids and J. Carbon for communicating the *ARG4* sequence before its publication. D. Maloney, M. Williamson and A. Blechl provided stimulating discussions and helpful suggestions on the manuscript. This work was supported by NIH grant GM 17317 awarded to S.F.

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Heresy of immanence

John Maddox

The Probability of God. By Hugh Montefiore.
SCM Press, 26-30 Tottenham Road, London: 1985. Pbk £6.95.

ON MATTERS of dogma the Anglican Church has an almost Unitarian god-of-your-choice flexibility, typified by the vicar in the 1960s revue "Beyond the Fringe" who was indistinguishable from a social worker and whose slogan was "Call me Bill; that's the kind of vicar I am". Hugh Montefiore, Anglican Bishop of Birmingham since 1978, is that kind of bishop.

The Probability of God is only marginally a theological work which, in that respect, has much in common with the opinions of the turbulent northern bishops such as the new incumbent at Durham, who is forever shocking the popular newspapers by saying that he doubts the doctrine of transubstantiation. The real purpose of the book is to demonstrate the immanence of God (or, more ecumenically, I suppose, some God) from the explanatory incompleteness of scientific theories, from cosmology to evolution.

To be frank (and fair), the book is a good and even entertaining read. Over the past few years, Montefiore has taken a serious and continuing public interest in matters such as the environment and arms control. He is both reasonable and earnest, willing to acknowledge that he does not know science at first hand, insistent nevertheless that it must be possible for an honest enquirer to get to the bottom of it (in which he is right) and, then, willing to take up the cudgels in the defence of what seems sanity. If there have to be bishops, nobody could ask for better.

The book is as reasonable as the man. On cosmology, for example, Montefiore does not seize on the big bang as proof that there is (or at least was) a Creator; that, he says, would be "to confuse secondary with primary causes". Instead, he follows the recent writings of Professor Paul C. Davies in emphasizing the numerical coincidences without which life as we know it would not have been possible — the small but not-too-small degree of anisotropy at the beginning that made it possible for galaxies to form; the particular ratio of gravitational and electromagnetic forces which ensures that all stars are not red dwarfs; the particular value of the coupling constant of the strong nuclear force which means that heavy elements (which we need in our enzymes) are stable but which is not so great that the post-big-bang ratio of hydrogen to helium would have been negligible.

Montefiore's refrain is typified by the declaration that "It seems remarkable that the possibility of a planet where life as we know it could evolve should depend upon

the precise value of this, the weak nuclear coupling constant". The anthropic principle, which has it that people can observe only a universe conducive to their own existence, is dismissed, but unconvincingly. Lovelock's Gaia hypothesis is a natural next: if the temperature on the surface of the Earth has been that which has been suitable for the survival of living things since the Precambrian, the ups and downs of the solar constant, or the variations of the chemical composition of the atmosphere notwithstanding, does it not follow that there must have been some intervention on behalf of us and our kind?

The texture of the two evolutionary chapters is different, even though Montefiore again begins by not taking the easy way out by letting everything hang on the belief that there has been too little time since the beginning of the Solar System for such a complicated set of living species as are now extant to have evolved. Montefiore argues instead from particular examples, but first asserts that science has made a dogma of natural selection. The rest is a collection of gee-whizz stories, "puzzle after puzzle". Why are our viscera the way they are? Why should *Drosophila* larvae be interposed between egg and adult? Why several independent and apparently successful attempts to evolve an eye? Popper's description of Darwinism as metaphysical (because it cannot be falsified) is mentioned, Popper's recantation is not.

In passing, Montefiore roundly castigates the Creationists, condemns Lysenko, concludes regretfully that there is no hope for Christians in Lamarck but plainly has a soft spot for Dr Ted Steele, the immunologist who caused a minor sensation a few years ago by claiming that immunological genetic changes may be inherited in the germ line. Mystifyingly, he also pats Rupert Sheldrake on the back, noting that the tendency for living cells to assemble in ordered ways can be explained if, indeed, there are "morphogenetic fields" which, for example, guide neurones to the connections with others that make them function properly.

Montefiore's God, therefore, is not a once and for all God, one who drew up the laws of physics and then left us to it, nor a "God of the gaps", one who may have intervened from time to time, but is instead an immanent God, embedded in the Universe and helping to make it tick from one instant to the next. By means of an argument reminiscent of Arthur Koestler's *Roots of Coincidence*, a tract in favour of the paranormal, Montefiore concludes that

the nature of the Universe makes the hypothesis of God probable, whereupon more familiar religious arguments lead to Christianity and all that. In passing (and at the outset), he rejects the notion that religion is a code by which people may distinguish ethical and moral behaviour from other kinds of behaviour, and which is therefore orthogonal to rational consideration of the Universe.

Although the cosmological part of Montefiore's argument would fall apart if the ambitions of particle physicists to unify the four forces of nature were to succeed, and demonstrate that the cosmic coincidences can be derived from the laws of physics, the flaw in the whole argument is not in the detail but in the grand design. Montefiore writes as if present scientific understanding of the Universe were complete, as if we shall always be stuck, for example, with the model of the big bang (in its contemporary form, no older than the discovery of the microwave background radiation). It would be an interesting exercise if somebody were to rewrite Montefiore's book at intervals of, say, five years; his natural theology (*pace* David Hume) would repeatedly be found to derive from different sets of circumstances.

Indeed, Montefiore's whole enterprise is more hazardous, and dangerous, than his reasonable tone implies. His immanent God, working in everything, qualifies him as a pantheist. One does not have to go quite as far as Hermann Bondi, whom Montefiore quotes as having described religion as a "dangerous and habit-forming evil", to be deeply suspicious of how pantheism could corrupt the process of scientific enquiry. At what point in the investigation of the evolution of the human eye, for example, will investigators feel free to chicken out, ascribing some parts of the process to natural selection and others to God? Montefiore says his is not the "God of the gaps", but it sounds a bit like the God of the unsolved problems.

Religious belief is common, even among scientists, and, in moderation, is at worst harmless. Many believers are content with what seems to them the sensible position that religious belief is neither dependent on nor even connected with the character of the Universe in which they live and carry out experiments. Others take the view that physical divine intervention ended with the big bang. Explanations of the real world that suppose there is an invisible hand somewhere, making sure that hidden purposes are fulfilled even in the face of the laws of physics are less common but, in their nature, more challenging. Montefiore's book, although honest to the point of saying that "the possibility that there is no God remains open" (but is "wildly improbable"), asks of its readers a concession to irrationality that most will find impossible to make. □

John Maddox is Editor of Nature.

Ringing the changes in chemistry

Robert Ramage

Comprehensive Heterocyclic Chemistry: The Structure, Reactions, Synthesis and Uses of Heterocyclic Compounds.

Chairmen of the editorial board Alan R. Katritzky and Charles W. Rees.
Pergamon: 1984. Eight volumes.
£1375, \$2200.

HETEROCYCLIC chemistry is a crucially important area of organic chemistry; it embraces a wide range of structural types and applications; and the pharmaceutical industry is, to a large extent, sustained by developments in the structure, reactivity and synthesis of heterocyclic systems. Pergamon's eight-volume series has these three themes as the unifying factor, which has enabled the principal editors and the volume editors to achieve an internally consistent presentation embodying coverage of a remarkable range of topics. The necessary editorial control exercised in the compilation of 154 chapters has not stifled the independent scholarship of the many contributing authors — their collective efforts have resulted in a work which can truly be considered to be "comprehensive".

The series is subdivided into six parts, and the approach adopted by the editors can best be illustrated by the general individual chapters on structure, reactivity and synthesis in Vols 2, 4, 5 and 7. These contributions are not only interesting in themselves but are excellent introductions to the subsequent, more specialized material in each section.

The editors have used Vol. 1 (which constitutes Part 1) to deal with general matters such as nomenclature, review literature, biosynthesis and applications of heterocyclic compounds, thus allowing the following sections to deal more with fundamental principles. In addition, the first volume treats an assortment of lesser known heterocyclic systems which could not easily be assimilated into the accounts of the main heterocyclic systems. Chapter 1.03 on the review literature of heterocycles includes an impressive yet accessible collection of a wide range of references, subdivided into synthesis, reactions and properties, while biological applications are succinctly discussed in chapters dealing with biosynthesis, toxicity, and pharmaceutical, agrochemical and veterinary products. Chapter 1.17 by K. Dimroth, on heterocyclic rings containing phosphorus, is especially impressive in content and organization and will allow chemists to appreciate more fully this less traditional area of the subject.

Part 2 covers six-membered ring systems incorporating one nitrogen atom (Vol. 2) and then oxygen, sulphur, or two or more nitrogen atoms (Vol. 3). The treatment of

these topics is consistent with the general editorial policy of logical development from structure through reactivity to synthesis. Highlights here are Chapter 2.03, a strategic discussion of synthesis of six-membered rings with varying degrees of unsaturation, and Chapters 2.05 and 2.06 which illustrate many facets of pyridine reactivity in monocyclic and bicyclic systems. Volume 2 will certainly provide good background reading in this general area, while the more specialized topics of Vol. 3 are treated in a clear, interesting manner. Together, these two volumes contain the fundamental chemistry required for understanding of many of the biologically important heterocyclic systems.

In Vol. 4 (Part 3 of the series), the approach to five-membered rings containing one hetero-atom is in accord with the pattern set out in the previous volumes. The introductory section (Chapters 3.01, 3.02 and 3.03) has been authoritatively written by C.W. Bird and G.W.H. Cheeseman, and there are useful accounts of the application of spectroscopic methods to structural problems of five-membered heterocycles, resonance and tautomerism. Pyrroles and their benzo derivatives are dealt with in some detail, and are followed by an excellent chapter by K.M. Smith on porphyrins and related compounds which examines in depth the synthesis of simple corrinoids and vitamin B₁₂. The treatment of furan and thiophene chemistry is both a critical and an extensive contribution.

Both Vols 5 and 6 (Part 4) include accounts of a remarkable range of heterocycles containing two or more hetero-atoms. Each class of ring system is treated consistently, and here are to be found

heterocyclic types ranging from the biologically important imidazoles and purines to the more specifically chemically interesting systems of importance in organic synthesis.

Volume 7 (Part 5) is used to collate the less voluminous chemistry of heterocyclic systems having small and large rings. The treatment of small-ring chemistry is instructive and well written, although the coverage of β -lactam antibiotics is of necessity rather too brief to be of more than general interest. The chemistry of seven-membered and larger rings is dealt with clearly and concisely, and crown ethers and cryptands are thoroughly reviewed by A.D. Hamilton who takes the subject from consideration of crystal structures to biological and synthetic applications of coordination of these macrocycles containing several hetero-atoms.

The final volume (and part) contains the index, helpfully divided into physical data, author, subject and ring. In addition, the work as a whole benefits from an ingenious reference code system which gives the reader easy access to original papers and source material such as books and reviews.

Comprehensive Heterocyclic Chemistry makes a unique contribution to the literature of organic chemistry. The references quoted are mainly post-1965, and together the eight volumes provide chemists, and biologists working at the chemical interface, with an impressive body of collected knowledge which is not available from any other source. Expensive though it is, chemistry libraries cannot afford to be without this series. □

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Bacterial diplomacy

Howard C. Berg

Motility and Taxis in Prokaryotes.

By A.N. Glagolev.
Harwood: 1984. Pp.279. \$126.

MODERN work on bacterial motility and taxis dates from the demonstration by Julius Adler (*Science* **166**, 1588-1597; 1969) that the bacterium *Escherichia coli* has specific chemoreceptors: proteins near its outer surface that bind particular amino acids or sugars, causing the cell to respond favourably to changes in its environment, even in the absence of transport or metabolism. This meant that one could study behaviour without having to worry much about energetics, using genetic tricks to unravel steps in the sensory-transductive pathway.

In the Soviet Union, interest in this field was sparked by Vladimir Skulachev, well known for his work on chemiosmotic proton circuits, following the discovery by Larsen, Adler, Gargus and Hogg (*Proc. natn. Acad. Sci. U.S.A.* **71**, 1239-1243;

1974) that bacterial flagella are powered, not by ATP, but rather by the high-energy intermediate of oxidative phosphorylation. Work in the Soviet Union, the United States and Japan has since shown that the immediate source of energy for flagellar rotation is, indeed, the protonmotive force. Alexei Glagolev, a protégé of Skulachev, now raises the Mitchellian banner and argues that $\Delta\mu\text{H}^+$ plays an important role in tactic sensing and signalling.

This is a research monograph, not a book for the casual reader. It begins with a compact survey of bioenergetics, summarizes work on the motile mechanics of flagellated cells and gliders, and then presents an extensive discussion of various forms of directed movement, including chemo-, photo-, thermo-, visco- and magneto-taxis. The book brings together the results of a number of experiments described previously by the author and his colleagues in a series of short papers appearing primarily in *FEBS Letters* and *FEMS Microbiology Letters*.

While many of the phenomena described in these papers and reviewed here are striking and warrant serious attention — experiments on the behaviour of long trichomes

of the gliding cyanobacterium *Phormidium uncinatum* are particularly elegant — I find myself sceptical of the work as a whole. A modern-day bioenergeticist comes armed with a number of chemical weapons (ionophores, uncouplers and inhibitors) with which I have had limited experience. How secure are the conclusions drawn from experiments in which such weaponry is applied? These reagents are not all highly specific. For example, the calcium ionophore A23187 has poor selectivity for Ca^{2+} over Mg^{2+} and can even exchange Na^+ for H^+ . The ATPase inhibitor DCCD reacts with alcohols and amines, not only with carboxyl groups in hydrophobic pockets. Furthermore, not all such reagents readily penetrate the outer membrane of gram-negative bacteria. The permeability properties of the outer membrane of *E. coli* are reasonably well understood, but what about *Ph. uncinatum*, *Rhodospirillum rubrum* or *Vibrio harveyi*, to name but a few of the organisms discussed here, or the archaebacterium *Halobacterium halobium*? These systems are complex. More than one function can be perturbed by chemical modification. How is the novice to judge the reliability of this approach when discussions are so brief?

My concern is reinforced by recent results obtained by the genetic approach. For example, by comparing the effects of the inhibitors DCCD and KCN on both phototaxis and motility in *H. halobium*, Glagolev argues that phototaxis in this organism is mediated by changes in $\Delta\mu\text{H}^+$. This bolsters his argument that sensory systems in bacteria might have evolved from a primitive protometer. However, as he readily acknowledges in his addendum (August 1983), the discovery that phototaxis in *H. halobium* is mediated by a slow-cycling rhodopsin distinct from either bacteriorhodopsin or halorhodopsin — see Spudich and Bogomolni *Nature* **312**, 509–513 (1984) — makes this interpretation highly unlikely. Or take the molecular model for chemotaxis (Fig.38), which includes (i) activation of cGMP by an attractant-receptor-MCP (methyl-accepting chemotaxis protein) complex, (ii) blockage by cGMP of the chemotaxis signal, comprised of a repellent-receptor-CheB (methyl-esterase) complex, which (iii) affects the flagella by binding to CheZ (a cytoplasmic

protein), thus liberating CheC (the flagellar switch) and allowing Ca^{2+} -dependent (possibly membrane potential-dependent) flagellar reversals. As far as I am aware, evidence for the involvement of either cGMP or Ca^{2+} in this process is equivocal, while mutants deleted for *cheB* and/or *cheR* (the methyltransferase) have normal excitation kinetics. So I find this model speculative in the extreme.

I therefore do not agree with the writer of the book's promotional blurb, who claims that this is "the first rigorous survey of the major new discoveries pertaining to bacterial behaviour". But I do find the book a lively, honest, even exuberant work,

full of interesting facts and figures, that carries the discussion of bacterial behaviour well beyond the narrow confines of the *E. coli*/*S. typhimurium* school.

One of the problems, regrettably, is that our Soviet colleagues have not had the benefit of much feedback from the West — one learns a great deal from peer review and the grapevine. Nor have we absorbed as much as we should of their bioenergetic expertise. The channels of communication should be strengthened. We need more bacterial diplomacy. □

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Only natural

L. Crombie

The Chemistry of Natural Products.

Edited by R.H. Thomson.

Blackie/Methuen: 1985. Pp.467.

£46, \$75.

ONE of the great barriers to a general appreciation of organic chemistry is the system of structural formulae employed by organic chemists. This is a pity, but understandable. The structural formula, with its dense information content, has evolved to its present form over a century and the practised organic chemist can take in at a glance an enormous amount of precise and detailed scientific information. Like any other language this pictogram system has to be learnt and practised, but once fluency is attained it is easy to use and, mathematical notation apart, is more universally accepted in science than any other language.

The Chemistry of Natural Products is full of such formulae, beautifully drawn and presented, and very helpful they are too. However, despite their plentiful use, the field of natural products is so large that a writer or editor must be very selective in the choice of topics to be covered in a single book. Professor Thomson has assembled a group of ten excellent authors to write about some of the principal sectors of the subject — carbohydrates, aliphatic and aromatic compounds, terpenes, steroids,

alkaloids, nucleosides, nucleotides and nucleic acids, amino acids, peptides and proteins, and porphyrins and related compounds. Even so, limitations have had to be imposed. Authors were asked to emphasize structure, chemistry and synthesis, and make only passing reference to biosynthesis. Additionally, their brief was to describe what has been happening in each of these areas during the past ten years. This of course places some restrictions on the readership which has to have an appreciable basic knowledge of the subject: for such people, however, the book is stimulating and valuable for keeping abreast of developments.

Undergraduates will probably find the chapters too terse and lacking in detailed explanation for direct use, but this should prove to be a useful book to their teachers for updating lecture notes. Postgraduates, especially those undertaking research in this area, will find it invaluable for acquiring a broader, up-to-date view of their subject. However, a price has had to be paid for this wide coverage in that, because of constraints on space, some authors have adopted a rather telegraphic style reminiscent of *Annual Reports of the Chemical Society* or certain of the *Specialised Periodical Reports*. For the purposes of this book, I would have preferred to have seen some reduction in the number of subjects covered and rather more relaxed and extended treatments.

Looking over these various topics, I sense that the contributors were necessarily assigned tasks of unequal difficulty. It is distinctly harder for authors of general sections such as "Aliphatic Compounds" or "Aromatic Compounds" to produce an exciting account because of the diversity of their material. On the other hand, more homogeneous topics — "Porphyrins and Related Compounds" or "Amino Acids, Peptides and Proteins" for example — make attractive reading and were doubtless more pleasurable to write. The book itself is a good example of a stout, well-produced product at an acceptable price. □

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Making waves — climbing parabolic sand dunes approaching Juniper Buttes, Snake River Plain, Idaho; wind direction is from lower left to upper right. The picture is reproduced from Wind as a Geological Process (on Earth, Mars, Venus and Titan), by Ronald Greeley and James D. Iversen. Publisher is Cambridge University Press, price is £35, \$59.50.

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ANNOUNCEMENTS

June meetings

5 June 1985, **Seminar on Laboratory Automation**, London, UK (Marketing Director, V.A. Howe & Co., 12-14 St Anne's Crescent, London SW18 2LS, UK)
 10-15 June 1985, **Bari International Conference on F.VIII/von Willebrand Factor-Biological and Clinical Advances**, Pizzomunno Vieste del Gargano, Italy (Dr N. Ciavarella, Hemophilia Centre, Policlinico, University, 70124 Bari, Italy)
 25-28 June 1985, **International Symposium on Forest Utilization of Municipal and Industrial Wastewater and Sludge**, Seattle, Washington, USA (K. Grier, College of Forest Resources AR-10, University of Washington, Seattle, WA 98195)

August meetings

24-27 August 1985, **International Conference on the Immunogenetics of Type I Diabetics and Autoimmune Thyroid Disease**, St. John's, Newfoundland, Canada (N.R. Farid, Prof. of Medicine, Chief-Division of Endocrinology and Metabolism, Memorial University of Newfoundland, St John's, Newfoundland, Canada)

September meetings

2-6 September 1985, **International Conference on Atmospheric Carbon Dioxide; Its Sources, Sinks and Global Transport**, Kandersteg, Switzerland (Ulrich Siegenthaler, Physics Institute, Sidlerstrasse 5, CH-3012 Bern, Switzerland)
 16-19 September 1985, **European Congress for Artificial Organs**, Athens, Greece (Prof. S. Raptis, M.D. P.O. Box 14127, 11510 Athens, Greece)
 16-20 September 1985, **International Meeting on Quantum Chemistry**, Strasbourg, France (Dr A. Veillard, Université Louis Pasteur, Institut Le Bel, 4 Rue Blaise Pascal, 67000 Strasbourg, France)
 17-19 September 1985, **International Symposium on Lyme Disease and Related Disorders**, Vienna, Austria (Dr G. Stanek, M.D. Hygiene Institute of the University, A-1095 Vienna, Austria)
 30 September-3 October 1985, **European Symposium on Hormones and Cell Regulation**, Strasbourg, France (Dr R.J.B. King, Imperial Cancer Research Fund Laboratories, Hormone Biochemistry Department, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK).

October meetings

3-4 October 1985, **International Meeting on Haemodynamics and Atherosclerosis**, Wellington, New Zealand (Dr W.E. Stehbens, Department of Pathology, Wellington Clinical School of Medicine, Wellington, 2, New Zealand)
 3-5 October 1985, **2nd Congress of the European Society of Magnetic Resonance in Medicine and Biology** (with Exhibition), Montreux, Switzerland (Dr M.A. Hopf, 1 route de Florissant CH-1206 Geneva, Switzerland)

8-10 October 1985, **International Congress and Exhibition for Biotechnology**, "Biotechnica", Hannover, West Germany (Deutsche Messe — und Ausstellungen — AG, Messegelände, D-3000, Hannover 82, FRG).

20-25 October 1985, **International Conference on Leukotrienes and Prostanoids in Health and Disease**, Jerusalem, Israel (Prof. U. Zor, Dept. of Hormone Research, Weizmann Institute of Science, Rehovot, Israel 76100)

21-24 October 1985, **International Conference on Pesticides Toxicity, Safety and Risk Assessment**, Lucknow, India (Dr T.S.S. Dikshith, General Secretary of the Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Post Box No. 80, Lucknow - 226001, India).

23-25 October 1985, **Congress of the International Society for Research on Civilization Diseases and Environment**, Budapest, Hungary (Congress Bureau MOTESZ, Budapest PO Box 32, H-11361, Hungary).

28 October-1 November 1985, **International Symposium on Source Term Evaluation for Accident Conditions**, Columbus Ohio, USA (M. Jankowski, Div. of Nuclear Safety, c/o Int. Atomic Energy Agency, Vienna International Centre, PO Box 100, A-1400 Vienna, Austria).

November meetings

18-21 November 1985, **Scientific Consensus Conference on methods for assessment of the cariogenic potential of foods**, Texas, USA (Dr Dominick P. DePaola, Chairman Consensus Conference Planning Committee, Dean, U.T.H.S.C.S.A., DS 7703 Floyd Curl Drive Antonio, Texas 78284, USA)

25 November-6 December 1985, **International Symposium on Optical and Electro-Optical Applied Science and Engineering**, Cannes, France (J. Prado, Cannes Conference Coordinator, ANRT-Association Nationale de la recherche Technique 101, Avenue Raymond Poincaré, 75116 Paris, France)

Appointments

President Reagan has announced his intention to nominate **Dr John H. Moore** as Deputy Director of the National Science Foundation (NSF). Dr. Moore has been Associate Director and Senior Fellow of the National Science Board, the policy making body of NSF since 1982. Dr Moore would succeed Donald Newton Langenberg.

The Minister of Agriculture, Fisheries and Food has appointed **Dr Robert N. Crossett**, Chief Scientist (Fisheries and Food) of the Ministry, to be a member of the Agricultural and Food Research Council.

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 4, Little Essex Street,
 London WC2R 3LF, UK

The KROC Foundation has endowed a \$1 million chair at UC-San Francisco to support biomedical research on rheumatic and other immunological diseases. The first holder of the Robert L. Kroc Chair in Rheumatic and Connective Tissue Diseases is **Edward J. Goetzl**, professor of medicine and director of the Division of Allergy and Immunology at UCSF.

Weizmann Institute chemist **Professor Zipora Shakked** has been named first incumbent of the Helena Rubinstein Chair in Structural Biology. Prof Shakked is a member of the Institute's Department of Structural Chemistry, applying X-ray crystallography methods to investigate compounds responsible for the expression of inherited traits.

Professor J. MacMillan, Professor of Organic Chemistry in the University of Bristol, has been appointed as Alfred Capper Pass Professor of Organic Chemistry in succession to Professor Whiting, who is retiring. Professor Whiting will be re-engaged part-time, as Professor of Organic Chemistry.

Dr J.D. Baum has been appointed to Bristol University's Chair in Child Health upon the retirement of Professor N.R. Butler at the end of July 1985.

Dr J.P. McGeehan, Senior Lecturer in Communications Engineering at the University of Bath, has been appointed to Bristol University's Chair in Communications Engineering.

Dr J.W.B. Bradfield, Consultant Senior Lecturer in Pathology at the University of Bristol, has been appointed to Bristol University's Chair in Histopathology.

Awards

The National Science Foundation is offering awards to women scientists and engineers wishing to become visiting professors at US academic institutions. Awards may range from one academic term to 24 months, full or part time, with the usual period being one year. For terms of eligibility and further information write to Visiting Professorships for Women Program, National Science Foundation, Washington, DC 20550.

Miscellaneous

The University of Essex is to offer the UK's first MSc Course in Aerosol Science and Technology from October this year. The one-year course will teach the theoretical and practical aspects of aerosol science and technology with specialist options in atmospheric chemistry and aerosols, and the physics of aerosols. For further information contact Irene Lally, Information Officer, University of Essex, Wivenhoe Park, Colchester, Essex.